

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

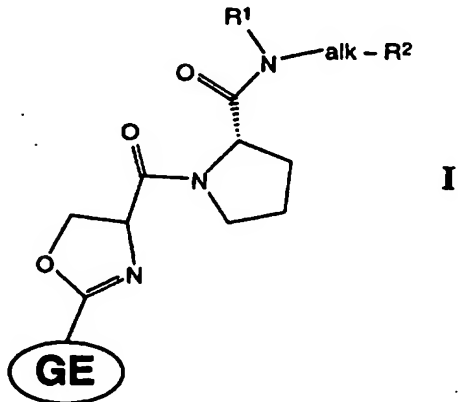
As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : <b>C07K 5/06, A61K 38/05 // C07K 7/56</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 96/24607</b> (43) International Publication Date: 15 August 1996 (15.08.96)</p>
<p>(21) International Application Number: <b>PCT/EP96/00407</b> (22) International Filing Date: 1 February 1996 (01.02.96) (30) Priority Data: 95101597.3 7 February 1995 (07.02.95) EP (34) Countries for which the regional or international application was filed: <b>GB et al.</b> (71) Applicant (for all designated States except US): <b>GRUPPO LEPETIT S.P.A. [IT/IT]; Via Roberto Lepetit, 8, I-20020 Lainate (IT).</b> (72) Inventors; and (75) Inventors/Applicants (for US only): <b>LOCIURO, Sergio [IT/IT]; Via Franco Tosi, 11, I-20143 Milano (IT). SENEI, Pierfausto [IT/IT]; Via Palestro, 24, I-20025 Legnano (IT). RESTELLI, Ermenegildo [IT/IT]; Via Rovello, 57, I-21040 Gerenzano (IT). CIABATTI, Romeo [IT/IT]; Via Brodolini, 15/A, I-20026 Novate Milanese (IT).</b> (74) Common Representative: <b>RAVIZZA, Claudio; Gruppo Lepetit S.P.A., Patent &amp; Trademark Dept., 34, Via Roberto Lepetit, I-21040 Gerenzano (IT).</b></p>		<p>(81) Designated States: <b>AU, CA, JP, KR, SI, US</b>, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i></p>
<p>(54) Title: <b>BASIC PROLINE-AMIDE DERIVATIVES OF GE 2270 AND GE 2270-LIKE ANTIBIOTICS</b></p>		
<p>(57) Abstract</p>		
<p>The present invention refers to basic amides derivatives of GE 2270 and GE 2270-like antibiotics of general formula (I), wherein the group GE represents the antibiotic core molecule. The amide derivatives of antibiotic GE 2270 of formula (I) are antimicrobial agents mainly active against gram positive bacteria.</p> <div style="text-align: center;">  <p style="text-align: right;"><b>I</b></p> </div>		

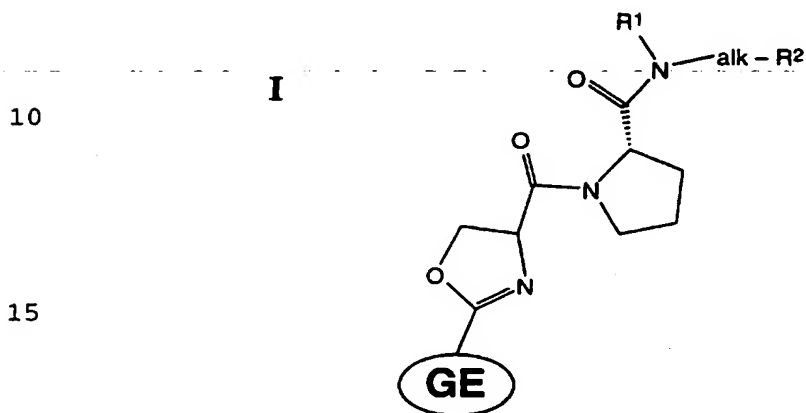
**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**BASIC PROLINE-AMIDE DERIVATIVES OF GE2270 AND  
GE2270-LIKE ANTIBIOTICS**

The present invention refers to basic amide  
5 derivatives of GE 2270 and GE 2270-like antibiotics of  
general formula I



20 wherein:

R<sup>1</sup> represents hydrogen, (C<sub>1</sub>-C<sub>4</sub>)alkyl or  
di(C<sub>1</sub>-C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene,

25 alk represents (C<sub>1</sub>-C<sub>4</sub>)alkylene or (C<sub>2</sub>-C<sub>5</sub>)alkylene-  
carbonyl,

R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

30 R<sup>3</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene,  
or di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene, and

R<sup>4</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-  
(C<sub>1</sub>-C<sub>4</sub>)alkylene or hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene,

35 or a five or six membered heterocycle ring  
containing one nitrogen atom and optionally a

further heteroatom selected from nitrogen and oxygen, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino and di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene;

or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring optionally containing a further heteroatom selected from oxygen and nitrogen, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>4</sub>)alkyl and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

R<sup>6</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-amino(C<sub>1</sub>-C<sub>4</sub>)alkylene and

R<sup>7</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-amino(C<sub>1</sub>-C<sub>4</sub>)alkylene

or a five or six membered heterocycle ring containing one or two heteroatoms selected from nitrogen and oxygen, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino and di(C<sub>1</sub>-C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene

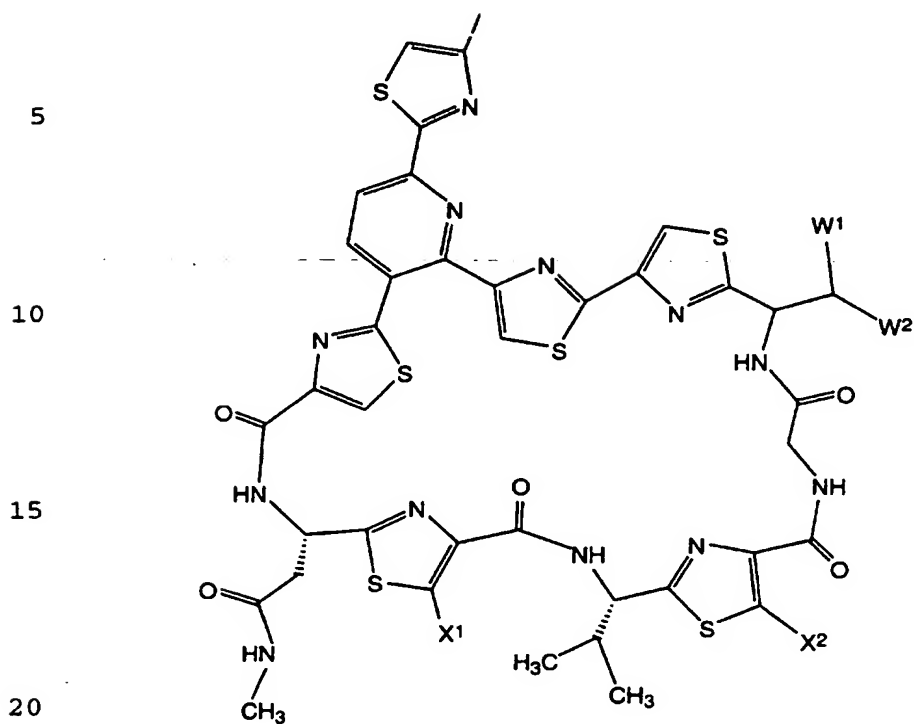
and the group of formula

30



represents the antibiotic core portion of formula

35



wherein:

W<sup>1</sup> represents phenyl

25 W<sup>2</sup> represents hydroxy

or both W<sup>1</sup> and W<sup>2</sup> represent methyl,

X<sup>1</sup> represents hydrogen or methyl,

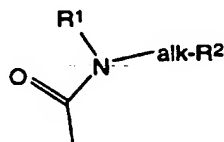
X<sup>2</sup> represents hydrogen, methyl or methoxymethylen,

30 with the proviso that when both W<sup>1</sup> and W<sup>2</sup> are methyl,  
then X<sup>1</sup> is methyl and X<sup>2</sup> is hydrogen,

or a pharmaceutical acceptable salt thereof.

35

The present invention refers also to the processes for preparing the compounds of formula I and to the carboxylic acid and protected carboxylic acid derivatives of the above compounds, i.e. the precursors of the compounds of formula I wherein the amidic group:



is substituted by the group  $-\text{COOY}$ , wherein Y represents hydrogen or  $(\text{C}_1-\text{C}_4)\text{alkyl}$ .

Antibiotic GE 2270 is prepared by culturing a sample of Planobispora rosea ATCC 53773 or a producing variant or mutant thereof and isolating the desired antibiotic substance from the mycelium and/or the fermentation broth. Planobispora rosea ATCC 53773 was isolated from a soil sample and deposited on June 14, 1988 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 Maryland, U.S.A., under the provisions of the Budapest Treaty. The strain has been accorded accession number ATCC 53773.

Antibiotic GE 2270 factor A is the main component of the antibiotic GE 2270 complex. Antibiotic GE 2270 factor A and Planobispora rosea ATCC 53773 are described in US Patent no. 5139778.

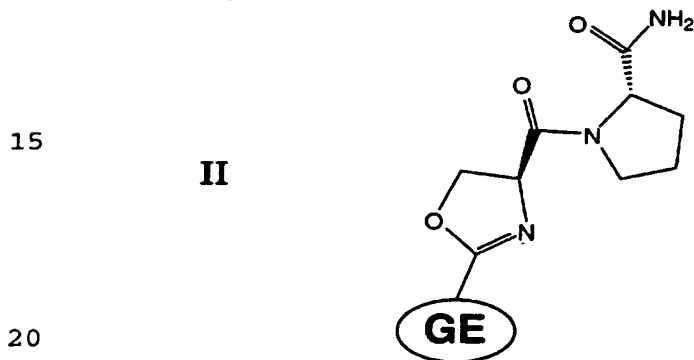
At present, a number of minor factors of antibiotic GE 2270 have been isolated, namely factors B<sub>1</sub>, B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub>, D<sub>1</sub>, D<sub>2</sub>, E and T disclosed in European Patent Application Publication no. 451486 and factor C<sub>2a</sub>

disclosed in European Patent Application Publication no. 529410. Also degradation products of GE 2270 factor A are known, namely factors A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> and H disclosed in US Patent no. 5139778.

5

Among these compounds, factor A, B<sub>2</sub>, C<sub>1</sub> and C<sub>2</sub> may be employed as suitable starting materials for preparing the compounds of the present invention.

10 The above factors may be represented by the following formula II:



wherein



25 is a group as above defined wherein

W<sup>1</sup> is phenyl and W<sup>2</sup> is hydroxy and

when X<sup>1</sup> is CH<sub>3</sub> and X<sup>2</sup> is CH<sub>2</sub>OCH<sub>3</sub>, factor A is determined;

when X<sub>1</sub> is CH<sub>3</sub> and X<sup>2</sup> is CH<sub>3</sub>, factor B<sub>2</sub> is determined;

when X<sub>1</sub> is CH<sub>3</sub> and X<sup>2</sup> is H, factor C<sub>1</sub> is determined; and

30 when X<sup>1</sup> is H and X<sup>2</sup> is CH<sub>2</sub>OCH<sub>3</sub>, factor C<sub>2</sub> is determined.

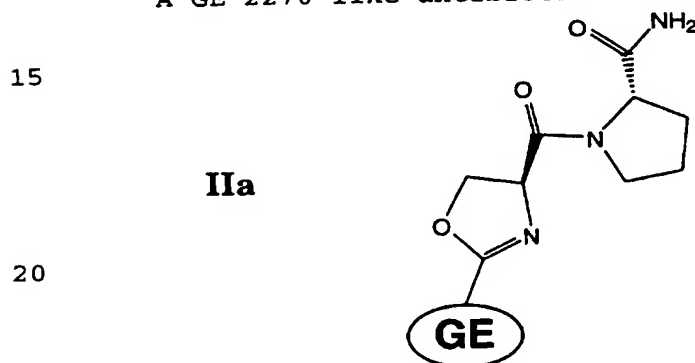
It should be noted that this formula does not correspond to the one disclosed in the above cited Patent Applications, which formula was assigned on the

35



basis of the physico-chemical data reported therein. As a matter of fact, further studies on the degradation products of the GE2270 factors (P. Tavecchia et al., Jour. of Antib., 47, no. 12 (1994), 1564-1567) have lead  
5 to the conclusion that the surmised aminoacid sequence was not correct, as the two aminoacids bearing the moieties X<sup>1</sup> and X<sup>2</sup> were actually in an opposite sequence in comparison with the formula previously reported; therefore the present formula II has been proposed for  
10 correctly representing the structure of antibiotic GE 2270.

A GE 2270-like antibiotic of formula IIa:



wherein **GE** is a group as above defined wherein

25 both W<sup>1</sup> and W<sup>2</sup> are methyl,  
X<sup>1</sup> is methyl and X<sup>2</sup> is hydrogen,

has been described by K. Shimanaka et al., Journal of Antibiotics, vol.47, pp. 668-674 (isolation, physico-chemical characteristics, antimicrobial activity) and  
30 vol. 47, pp. 1153-1159 (structure elucidation); both these articles are herein incorporated by reference.

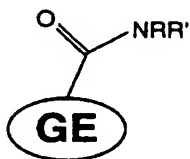
Said GE 2270-like antibiotic, named amythiamicin factor A, has been isolated from the fermentation broth of Amycolatopsis sp. MI481-42F4, which strain has been  
35 deposited in the National Institute of Bioscience and

Human-Technology, Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-12739.

The fermentation of Amycolatopsis sp. MI481-42F4 is conducted according to conventional methodologies in conventional nutrient medium; amythiamicin factor A shows antimicrobial activity against gram positive bacteria. Also this compound may suitably be employed as starting material for the process of the present invention.

In the following of the present specification, with the wording "GE 2270 starting material" is intended any suitable factor of antibiotic GE 2270, such as factor A, B<sub>2</sub>, C<sub>1</sub> and C<sub>2</sub>, as well as amythiamicin factor A.

Furthermore, amide derivatives of GE 2270 derivatives of general formula



wherein the group **GE** is as defined in formula II and R and R' have a plurality of meanings, are described in European Patent Application Publication No. 565567 (also in this case, for the reasons set forth before, the disclosed structure of the core portion is incorrect).

As evident, the above amide derivatives of GE 2270 differ from the compounds of the present invention in that the two terminal heterocycle rings of the GE2270 molecule (i.e. the oxazoline and the proline rings) are not present in the above general formula.

In the present description, the terms used above in defining the meanings of the substituents are intended to have the meanings commonly assigned to them in the art. Accordingly:

- 5 (C<sub>1</sub>-C<sub>4</sub>)alkyl represents a linear or branched hydrocarbon moiety containing 1, 2, 3 or 4 carbon atoms such as:

-CH<sub>3</sub>,  
-CH<sub>2</sub>-CH<sub>3</sub>,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>,  
10 -CH-(CH<sub>3</sub>)<sub>2</sub>,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>,  
-CH(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>3</sub>,  
-CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>3</sub>,  
-C-(CH<sub>3</sub>)<sub>3</sub>;

- 15 (C<sub>1</sub>-C<sub>4</sub>)alkylene represents a bifunctional linear or branched hydrocarbon moiety containing 1, 2, 3 or 4 carbon atoms such as:

-CH<sub>2</sub>-,  
20 -CH<sub>2</sub>-CH<sub>2</sub>-,  
-CH(CH<sub>3</sub>)-  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-,  
-CH(CH<sub>3</sub>)-CH<sub>2</sub>-,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-,  
25 -CH(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>2</sub>-,  
-CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>2</sub>-,  
-C(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-;

(C<sub>1</sub>-C<sub>4</sub>)alkylenecarbonyl represents a bifunctional carbonylic moiety containing 2 to 5 carbon atoms, such as:

30 -CH<sub>2</sub>-CO-,  
-CH<sub>2</sub>-CH<sub>2</sub>-CO-,  
-CH(CH<sub>3</sub>)-CO-,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-,  
-CH(C<sub>2</sub>H<sub>5</sub>)-CO-,  
35 -CH(CH<sub>3</sub>)-CH<sub>2</sub>-CO-,

-CH(C<sub>2</sub>H<sub>5</sub>)-CH<sub>2</sub>-CO-,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-,  
-CH(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>2</sub>-CO-,  
-C(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CO-;

5 hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene represents a linear or branched alcanolic moiety of from 1 to 4 carbon atom, such as:

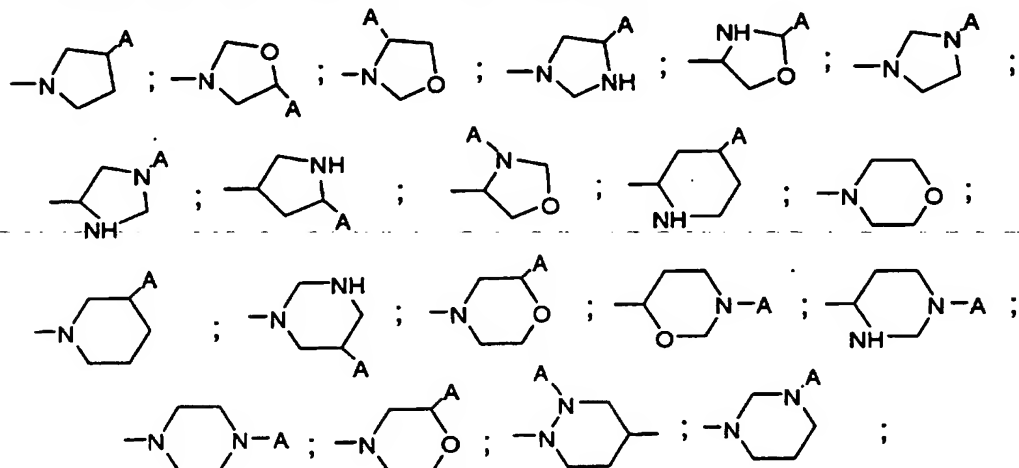
-CH<sub>2</sub>-OH,  
-CH<sub>2</sub>-CH<sub>2</sub>-OH,  
-CH(CH<sub>3</sub>)-OH  
10 -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-OH,  
-CH(CH<sub>3</sub>)-CH<sub>2</sub>-OH,  
-CH<sub>2</sub>-CH(CH<sub>3</sub>)-OH  
-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-OH,  
-CH(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>2</sub>-OH,  
15 -CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>2</sub>-OH,  
-CH<sub>2</sub>-CH<sub>2</sub>-CH(CH<sub>3</sub>)-OH  
-C(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-OH;

di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, is an amino moiety substituted with  
20 two linear or branched alkyl groups containing 1, 2, 3  
or 4 carbon atoms such as:

-N-(CH<sub>3</sub>)<sub>2</sub>,  
-N(CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>3</sub>)<sub>2</sub>,  
25 -N(CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>)<sub>2</sub>,  
-N(CH<sub>3</sub>)[CH-(CH<sub>3</sub>)<sub>2</sub>],  
-N(CH<sub>2</sub>-CH<sub>3</sub>)[CH-(CH<sub>3</sub>)<sub>2</sub>],  
30 -N(CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>)(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),  
-N(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>)<sub>2</sub>,  
-N(CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>)[CH-(CH<sub>3</sub>)<sub>2</sub>];

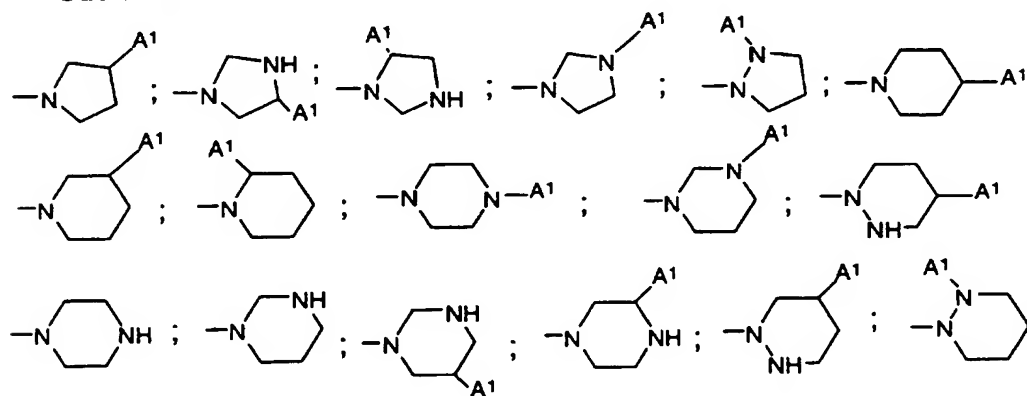
35

a five or six membered heterocycle ring according to the meanings of R<sup>2</sup> or R<sup>5</sup> is an heterocycle ring such as:



wherein A represents hydrogen or hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene when referring to the substituent "R<sup>2</sup>" or A represents only hydrogen when referring to the substituent "R<sup>5</sup>";

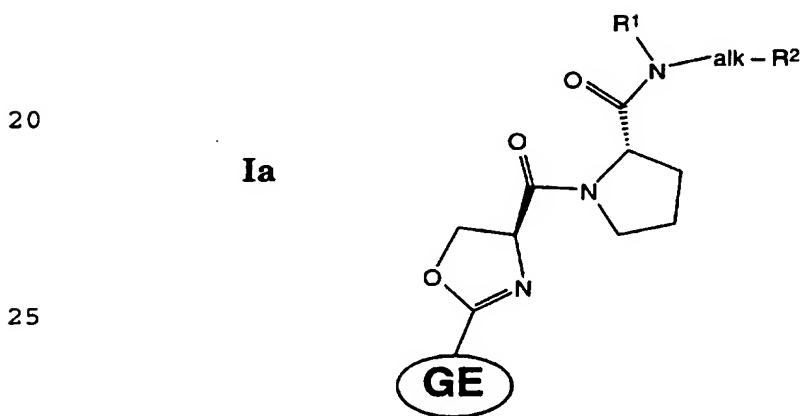
a five or six membered heterocycle ring formed by the moieties R<sup>1</sup> and alk-R<sup>2</sup> together, is an heterocycle ring such as:



wherein A<sup>1</sup> represents hydrogen or the optional substituents of the heterocycle ring as set forth before.

By comparing the above formulas I and II, it appears that GE 2270 factors naturally occur with a determined chirality of the molecule; according to the present invention, the compounds of formula I may be obtained with both the chiralities, with respect to the bond between the oxazoline and the proline rings. Although in most cases, the antimicrobial activity of the two epimers (either of the starting materials or of the compounds of the invention) is almost the same, in some cases, against particular strains (e.g. streptococci), it has been observed a slightly higher antimicrobial activity for those compounds having the chirality corresponding to the natural one.

Thus, a group of preferred compounds of the invention are those compounds of general formula Ia



Another group of preferred compounds are those compounds of formula I or Ia wherein the group **GE** is such that W<sup>1</sup> is phenyl, W<sup>2</sup> is hydroxy, X<sup>1</sup> is methyl, X<sup>2</sup>

is methoxymethylen and R<sup>1</sup>, alk and R<sup>2</sup> are as defined in formula I.

A further group of preferred compounds are those  
5 compounds of formula I or Ia wherein the group **GE** is as defined in formula I and

R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>4</sub>)alkyl,

10 alk represents (C<sub>1</sub>-C<sub>4</sub>)alkylene or (C<sub>2</sub>-C<sub>5</sub>)alkylene-carbonyl,

R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

R<sup>3</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl and

15 R<sup>4</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene,

or a five or six membered heterocycle ring containing one or two nitrogen atoms, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl  
20 and hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene;

or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring optionally containing a further nitrogen atom,

25 optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkyl and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

30 R<sup>6</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl and

R<sup>7</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-amino(C<sub>1</sub>-C<sub>4</sub>)alkylene

or a five or six membered heterocycle ring containing one or two heteroatoms selected from  
35 nitrogen and oxygen.

A further group of preferred compounds are those compounds of formula I or Ia wherein the group **GE** is as defined in formula I and

- 5  
R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>2</sub>)alkyl,  
  
alk represents (C<sub>1</sub>-C<sub>3</sub>)alkylene or (C<sub>2</sub>-C<sub>3</sub>)alkylene-carbonyl,  
10  
R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein  
R<sup>3</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl and  
R<sup>4</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-(C<sub>1</sub>-C<sub>2</sub>)alkylene,  
15 or a five or six membered heterocycle ring containing one or two nitrogen atoms, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl and hydroxy(C<sub>1</sub>-C<sub>2</sub>)alkylene;  
  
20 or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring optionally containing a further nitrogen atom, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-  
25 (C<sub>1</sub>-C<sub>2</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkyl and  
R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein  
R<sup>6</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl and  
R<sup>7</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkyl-amino(C<sub>1</sub>-C<sub>2</sub>)alkylene  
30 or a five or six membered heterocycle ring containing one or two heteroatoms selected from nitrogen and oxygen.



Particularly preferred compounds are those compounds of formula I or Ia wherein the group **GE** is such that W<sup>1</sup> is phenyl, W<sup>2</sup> is hydroxy, X<sup>1</sup> is methyl, X<sup>2</sup> is methoxy-  
5 methylene and

R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>2</sub>)alkyl,

alk represents (C<sub>1</sub>-C<sub>3</sub>)alkylene,

10

R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

R<sup>3</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl and

R<sup>4</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-  
(C<sub>1</sub>-C<sub>2</sub>)alkylene,

15

or a five or six membered heterocycle ring containing one or two nitrogen atoms, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl and hydroxy(C<sub>1</sub>-C<sub>2</sub>)alkylene;

20 or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring optionally containing a further nitrogen atom, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-  
25 (C<sub>1</sub>-C<sub>2</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkylene and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

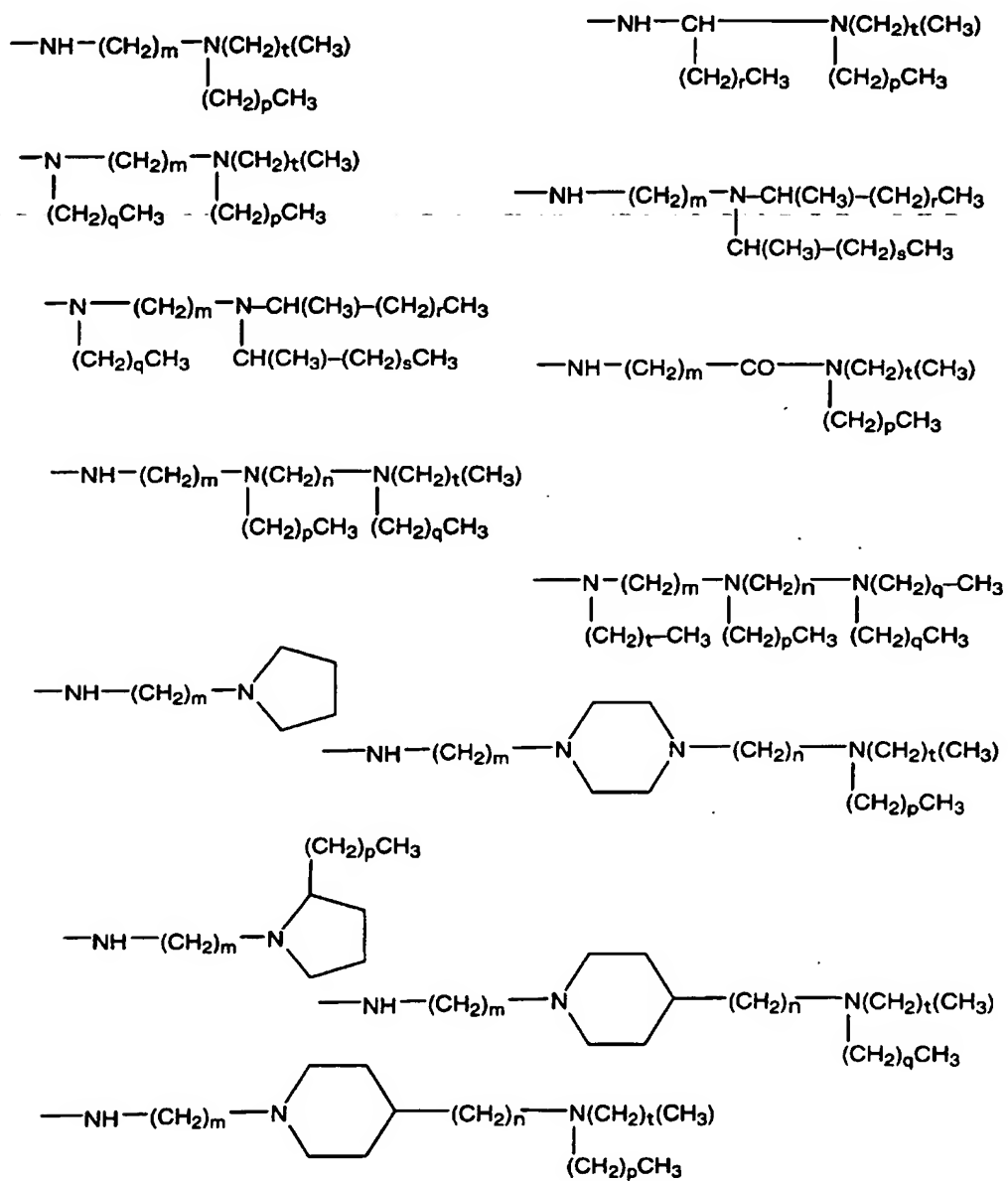
R<sup>6</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl and

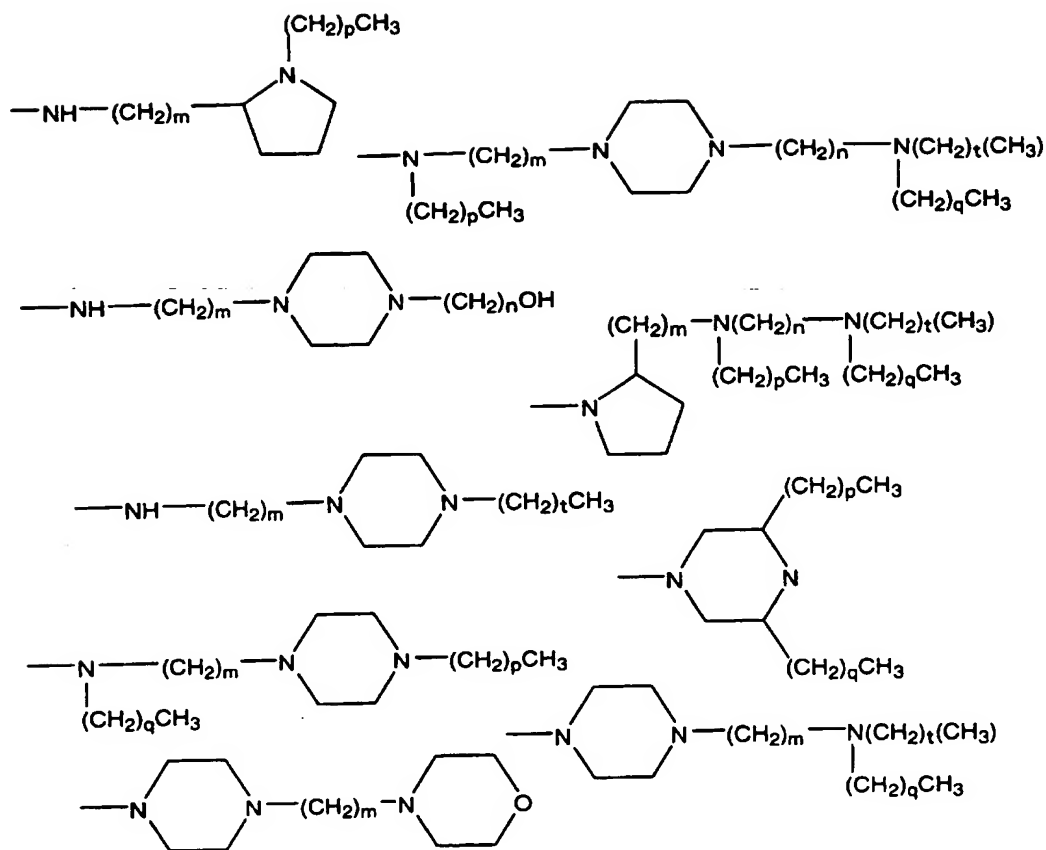
R<sup>7</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkyl-  
30 amino(C<sub>1</sub>-C<sub>2</sub>)alkylene

or a five or six membered heterocycle ring containing one or two heteroatoms selected from nitrogen and oxygen.

35

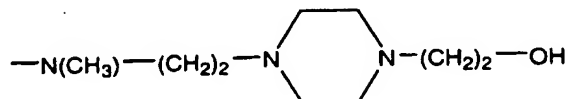
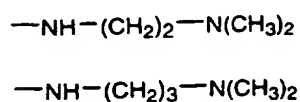
Examples of  $-N(R^1)alkR^2$  groups, as defined in formula I, are the following:

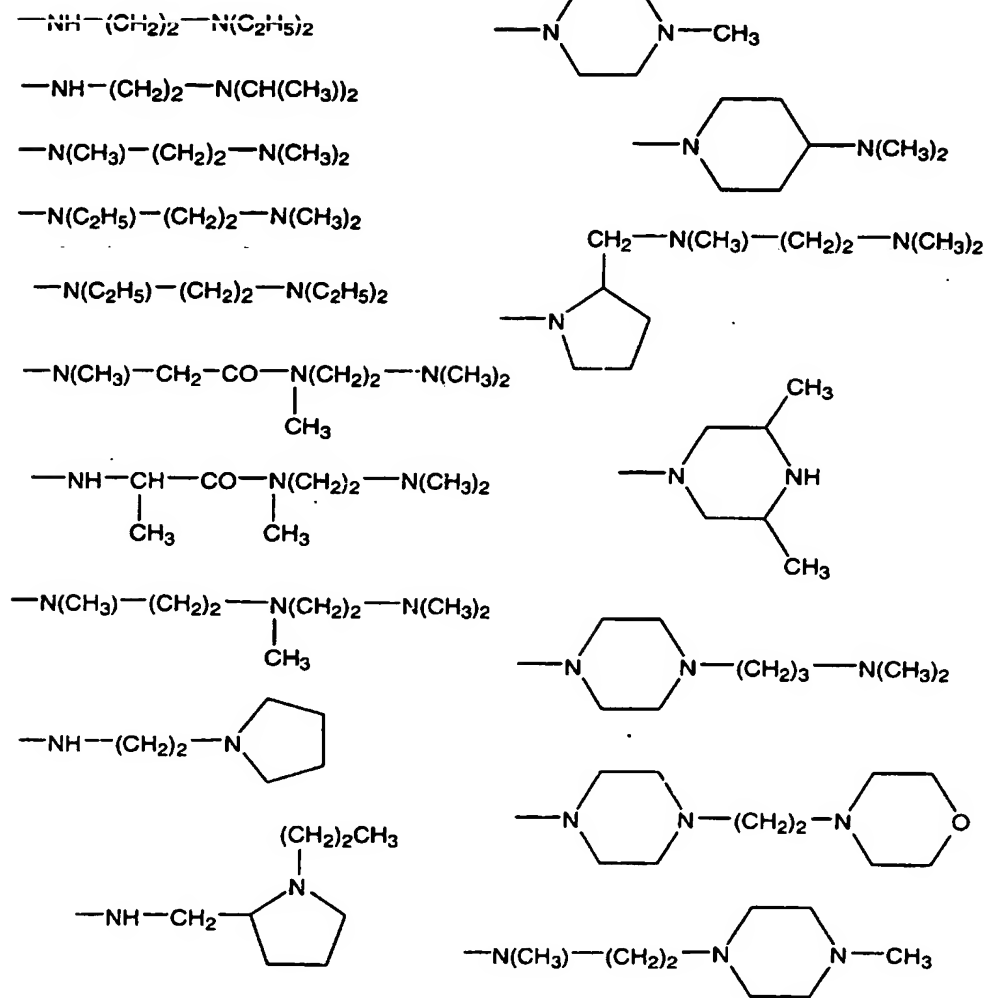




- 25 wherein:  
 m and n = 1, 2, 3 or 4;  
 p, q and r = 0, 1, 2 or 3  
 s = 0 or 1.

- 30 Preferred examples of  $\text{—N(R}^1\text{)alkR}^2$  groups are the following:





30

The compounds of the invention can form salts according to conventional procedures.

35

In particular, those compounds of formula I wherein the group  $-N(R^1)alkR^2$  contains further amine functions can form acid addition salts.

5 Preferred addition salts of the compounds of this invention are the pharmaceutically acceptable acid addition salts.

With the term "pharmaceutically acceptable acid addition salts" are intended those salts with acids  
10 which from biological, manufacturing and formulation standpoint are compatible with the pharmaceutical practice as well as with the use in the animal growth promotion.

15 Representative and suitable acid addition salts of the compounds of formula I include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic,  
20 trichloroacetic, succinic, citric, ascorbic, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, dodecylsulfonic (estolic), benzenesulfonic, sorbic,  
25 picric, benzoic, cinnamic acid and the like.

The transformation of the free amino or non-salt compounds of the invention into the corresponding addition salts, and the reverse, i.e. the transformation  
30 of an addition salt of a compound of the invention into the non-salt or free amino form, are within the ordinary technical skill and are encompassed by the present invention. The only precaution is to avoid solutions with pH lower than 4-5 when preparing the addition salt  
35 (for avoiding the opening of the oxazolinic ring) and

solutions with a pH higher than 8-9 when freeing the base (for avoiding epimerization on the chiral center).

For instance, a compound of formula I can be  
5 transformed into the corresponding acid addition-salt by dissolving the non-salt form in an aqueous solvent and adding a slight molar excess of the selected acid. The resulting solution or suspension is then lyophilized to recover the desired salt. Instead of lyophilizing, in  
10 some instances, it is possible to recover the final salt by extraction with an organic solvent, concentration to a small volume of the separated organic phase and precipitation by adding a non-solvent.

15 In case the final salt is insoluble in an organic solvent where the non-salt form is soluble, it may be recovered by filtration from the organic solution of the non-salt form after addition of the stoichiometric amount or a slight molar excess of the selected acid.

20

The non-salt form can be prepared from a corresponding acid salt dissolved in an aqueous solvent which is then neutralized to free the non-salt form. This is then recovered for instance by extraction with  
25 an organic solvent or is transformed into another acid addition salt by adding the selected acid and working up as above.

A common desalting procedure may be employed when,  
30 following the neutralization, desalting is necessary.

For example, column chromatography on controlled pore polydextrane resins (such as Sephadex LH 20) or silanized silica gel may be conveniently used. After eluting the undesired salts with an aqueous solution,  
35 the desired product is eluted by means of linear

gradient or step-gradient of a mixture of water and a polar or apolar organic solvent, such as acetonitrile/water from 50:50 to about 100% acetonitrile.

5

As is known in the art, the salt formation either with pharmaceutically acceptable acids or non-pharmaceutically acceptable acids may be used as a convenient purification technique. After formation and  
10 isolation, salt form of a compound of formula I can be transformed into the corresponding non-salt or into a pharmaceutically acceptable salt.

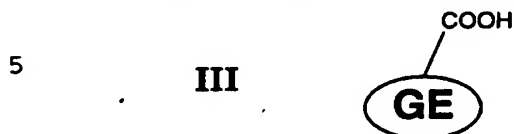
In some instances the acid addition salt of a  
15 compound of formula I is more soluble in water and hydrophilic solvents and has an increased chemical stability. Good solubility and stability in water or hydrophylic solvents of an active compound are in general appreciated in the art, for the preparation of  
20 suitable pharmaceutical compositions for the administration of the medicament.

However, in view of the similarity of the properties of the compounds of formula I and their salts, what is  
25 said in the present application when dealing with the biological activities of the compounds of formula I applies also to their pharmaceutically acceptable salts, and viceversa.

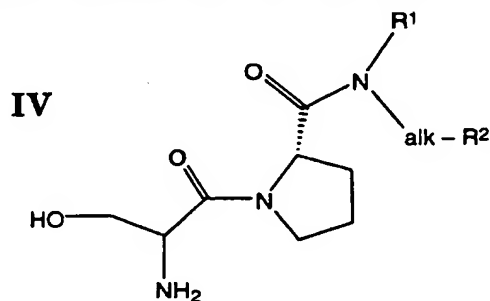
30

35

A suitable method for preparing the compounds of the invention (hereinafter defined as "Method A") comprises reacting a compound of formula III

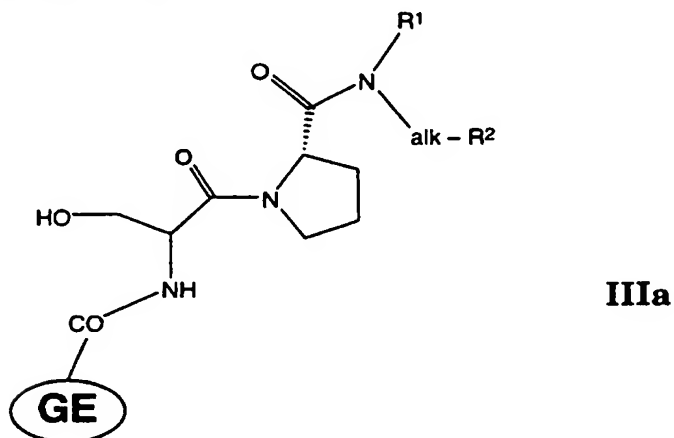


wherein the group **GE** is as defined in formula I, with a suitable serinamide of formula IV:



20                    wherein R<sup>1</sup>, alk and R<sup>2</sup> are as in formula I, in an inert aprotic organic solvent in the presence of a condensing agent;

b) cyclizing the serine moiety of the obtained compound of formula IIIa





with a suitable cyclizing reactant, in order to obtain the desired compound of formula I.

According to method A, the chirality of the final compound is determined by the chirality of the serinamide reactant employed, with retention of the configuration of the serine chiral center. Thus, for obtaining the amide derivatives with a chirality corresponding to the natural one, L-serinamides shall be employed.

10

Inert organic aprotic solvents useful for the condensation reaction according to method A are those solvents which do not unfavorably interfere with the reaction course and are capable of at least partially solubilizing the antibiotic starting material.

15

Examples of said solvents are organic amides, ethers of glycols and polyols, phosphoramides, sulfoxides and mixtures thereof. Preferred examples are: dimethylformamide, dimethoxyethane, hexamethylphosphoramide, dimethylsulfoxide, dioxane, and mixtures thereof. Preferably, dimethylformamide (DMF) is employed.

20

The condensing agent in the present method is one suitable for forming amide bonds in organic compounds and in particular in peptide synthesis.

25

Representative and preferred examples of condensing agents are (C<sub>1</sub>-C<sub>4</sub>)alkyl, phenyl or heterocyclic phosphorazidates such as, diphenyl-phosphorazidate (DPPA), diethyl-phosphorazidate, di(4-nitrophenyl)-phosphorazidate, dimorpholyl-phosphorazidate and diphenylphosphorochloridate or benzotriazol-1-yl-oxy-tripyrrolidinophosphoniumhexa-fluorophosphate (PyBOP). The preferred condensing agent is DPPA.

30

The condensing agent is generally employed in a slight molar excess, such as from 1.1 to 1.5; preferably

35

the molar excess of condensing agent is 1.2 times the amount of antibiotic GE 2270 starting compound.

According to the present method, the serinamide of  
5 formula IV is normally used in a slight molar excess.

In general, a 1 to 1.5 fold molar excess is used, while a 1.2 fold molar excess is preferred.

For the amidation to proceed, it is necessary that the serinamide of formula IV be capable of forming a  
10 salt with the carboxy function of the antibiotic starting material. As this could require the use of a higher amount of the serinamide, in such a case it is convenient to add a salt-forming base to the reaction mixture, at least in an equimolecular amount, and  
15 preferably a 2 to 3 fold molar excess, with respect to the antibiotic starting material.

Examples of said salt-forming bases are tertiary organic aliphatic or alicyclic amines such as trimethylamine, triethylamine (TEA), N-methyl  
20 pyrrolidine or heterocyclic bases such as picoline, and the like.

In addition, the serinamide of formula IV may also conveniently be introduced in the reaction medium as a corresponding acid addition salt, such as hydrochloride,  
25 trifluoroacetate, and the like. In fact, at least in some instances, the use of the salified serinamide of formula IV, which is then freed in situ with the above mentioned bases, is preferred, particularly when the salt is more stable than the corresponding free amine.  
30 In this case, at least a double molar proportion and preferably a 2 to 3 fold molar excess of a base capable of freeing the serinamide of formula IV from its salts, is used. Also in this case, the suitable base is a tertiary organic aliphatic or alicyclic amine like those  
35 exemplified above, preferably TEA.

The reaction temperature will vary considerably depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures from 0°C to room temperature, preferably starting at about 0°C and allowing the mixture to reach room temperature during the reaction.

Also the reaction time varies considerably depending on the other reaction parameters; in general the condensation is completed in about 5-24 h.

Generally, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art. On the basis of the results of these assays a man skilled in the art will be able to evaluate the reaction course and decide when to stop the reaction and start working up the reaction mass according to known per se techniques. For instance the reaction mixture may be poured into an aqueous basic solution for precipitating the compound of formula IVa as an addition salt. The basic solution should have a pH suitable for precipitating the salt of the desired compound, without modifying its chemical structure. In general, the pH ranges from 8 to 10, and is obtained with an aqueous solution of an inorganic base, such as alkali or alkaline-earth metal hydroxides, carbonates, bicarbonates, and the like. The compound of formula IVa is obtained as a crude, after filtration and evaporation of the above basic solution, as the purification step is preferably accomplished after the cyclization reaction. However, when a purified product is desired, the known per se separation and purification techniques may be employed, which include, for instance, extraction with solvents, precipitation by pH modification, precipitation by addition of non-solvents, etc., in

conjunction with further separations and purifications by column chromatography.

- 5        Step b) of the present process, i.e. the serine-oxazoline cyclization is performed according to methods known per se in the art.

          According to a preferred embodiment, the compound of formula IIIa is reacted with methoxycarbonylsulfamoyl-  
10        triethylammonium hydroxide, inner salt (Burgess reagent), and the reaction mixture is then refluxed for obtaining the oxazoline cyclization.

          More in detail, the obtained compound of formula  
15        IIIa is reacted with an excess (about 3:1 to 15:1) of Burgess reagent, in the presence of an organic aprotic oxygenated solvent, for obtaining the corresponding sulfamoyl ester of the Burgess reactant.

20        Examples of organic aprotic oxygenated solvents are saturated linear or cyclic ethers or glycol ethers. Preferred examples of said solvents are tetrahydrofuran (THF), dioxane. Optionally chlorinated solvents may also be added to the reaction mixture, such as  
25        dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), chloroform, for increasing the solubility of the reactants.

          Optionally, a base may also be added to the reaction mixture, for avoiding undesired side-reactions. Examples  
30        of suitable bases are tertiary organic aliphatic or alicyclic amines such as trimethylamine, triethylamine (TEA), N-methyl pyrrolidine or heterocyclic bases such as picoline, and the like; preferably TEA is employed.

The reaction temperature will vary considerably depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures of from 18°C to 30°C, preferably at room temperature.

Also the reaction time varies considerably depending on the other reaction parameters; in general the reaction is completed in about 4 to 20 hours.

Generally, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art.

After the reaction is completed, a secondary or tertiary alcohol is added to the reaction mixture, for quenching the reaction. Said alcohol should be able to react with the unreacted Burgess reactant and be transformed in olefinic compounds, preferably low boiling olefines. Thus, a secondary or tertiary (C<sub>3</sub>-C<sub>5</sub>) alcohol may suitably be employed, such as isopropanol, tert-butanol, 1-methyl-propanol, 1,1-dimethyl-propanol, 1,2-dimethyl-propanol, 1-ethyl-propanol; preferably, isopropanol is employed.

The reaction mixture is then refluxed for cyclizing the oxazoline. Time and temperature of the reflux will vary mainly depending on the solvents present in the reaction mixture. For instance, if low boiling solvents (e.g. alcohols, chlorinated solvents) are removed before refluxing, higher reflux temperatures are obtained. Thus, depending on the type of solvents present in the refluxing mixture, the temperature will vary from 50°C to 80°C. In general, as the higher the reflux temperature, the shorter the time, the reflux time will accordingly vary from 20 to 5 hours.

Also in this case, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art. On the basis of the results of these assays a man skilled in the art will be able to decide when to stop the reflux and start working up the reaction mass according to known per se techniques, which include, as above, extraction with solvents, precipitation by pH modification, precipitation by addition of non-solvents, etc., in conjunction with further chromatographic separations and purifications techniques, such as flash chromatography (e.g. on silica gel using dichloromethane/methanol mixtures as eluent), reverse phase chromatography or chromatography on neutral aluminium oxide (using dichloromethane/methanol mixtures as eluent).

The starting material of formula III wherein the group **GE** is such that W<sup>1</sup> is phenyl, W<sup>2</sup> is hydroxy, X<sup>1</sup> is methyl and X<sup>2</sup> is methoxymethylene, corresponding to antibiotic GE 2270 factor A<sub>3</sub>, and the hydrolysis process for preparing it, are disclosed in US Pat. No. 5139778.

Generally, the above mentioned hydrolytic conditions involve the use of mixtures of buffered or unbuffered aqueous acid media and polar organic solvents. The reaction temperature varies depending on factors such as the strength and the concentration of the acid employed, and is generally comprised between -10°C and 90°C. Also the reaction time varies considerably depending on parameters such as the temperature, the acid strength and its concentration; generally, it may vary from a few minutes to several hours.

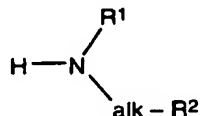
In general, when milder hydrolysis conditions are employed, e.g. shorter reaction time and lower temperature or lower acid strength or concentration,

antibiotic GE 2270 factor A<sub>1</sub> is normally obtained, while stronger hydrolysis conditions yield antibiotic GE 2270 factor A<sub>2</sub>. To obtain antibiotic GE 2270 factor A<sub>3</sub>, still more drastic hydrolysis conditions are necessary. Factor A<sub>2</sub> may also be converted into factor A<sub>3</sub> by basic hydrolysis with diluted alkali.

By following the above procedure, but starting from GE 2270 factor B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub> or amythiamicin factor A instead of GE2270 factor A, the respective starting materials of formula III are obtained.

The serinamide of formula IV is prepared according to known per se techniques of peptide synthesis, described in a number of references books like E. Gross and J. Meienhofer "The Peptides", Vol. 3, Academic Press, New York, 1981 and M. Bodanszky and A. Bodanszky "The Practice of Peptide Synthesis, Springer-Verlag, Berlin Heidelberg, 1984.

As a general procedure, a N-protected L-proline is first reacted with the desired amine of formula IVa



IVa

wherein R<sup>1</sup>, alk, and R<sup>2</sup> are as defined in formula I; the obtained L-prolinamide is then deprotected and reacted with a N-protected serine, to give the desired N-protected starting material. As said above, when amide derivatives of formula I with a chirality corresponding to the natural one are desired, L-serinamides shall be

employed; accordingly, the prolinamide shall be reacted with N-protected L-serine.

As known in the art, the amidation reactions may either be performed in the presence of a condensing agent (e.g. phosphorazidates such as diphenylphosphorazidate, DPPA) or the N-protected amino acid may be reacted in the form of an activated ester (such as pentafluorophenyl, N-hydroxysuccinimide or 1-hydroxybenzothiazole ester)

10 The protecting group employed in both steps of the above described process are those generally employed in peptides synthesis. Preferably, the N-protection of serine is performed with protecting group which are easily removable under acid or neutral hydrolytic  
15 conditions, such as t-butoxycarbonyl (BOC) or benzyloxycarbonyl (cbz).

Preferably, the N-deprotection of the serinamide is performed only short before the amidation reaction with the GE2270 starting material, so to avoid the formation  
20 of undesired side products.

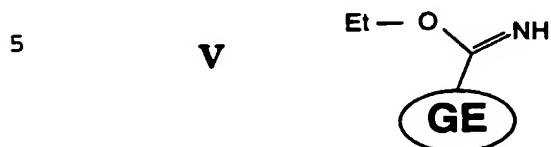
The amine of general formula IVa is either a commercially available compound or is prepared according to known per se techniques, described in a number of  
25 references books, such as "Comprehensive Organic Synthesis, vol. 8, 1991, Pergamon Press".

30

35



Another method (hereinafter defined as "Method B") for preparing the compounds of the invention is to react a compound of formula V



10                      wherein the group **GE** is as defined in formula I, with a serinamide of formula IV as above defined, in a protic organic solvent.

15                      Also in this case as in method A, the chirality of the final compound is determined by the chirality of the serinamide reactant employed, with retention of the configuration of the serine chiral center.

20                      Preferred protic organic solvents are those solvents which do not unfavourably interfere with the reaction course and are capable of at least partially solubilizing the antibiotic starting material. Preferred examples of such solvents are (C<sub>1</sub>-C<sub>4</sub>)alcohols, such as methanol, ethanol, propanol, iso-propanol, butanol, iso-butanol and mixtures thereof.

25                      Preferably, also minor amounts of an aprotic organic solvent are added, for increasing the solubility of the GE 2270 starting material; preferred solvents are in this case chlorinated solvents, particularly preferred  
30                      being dichloromethane.

35                      Furthermore, as the serinamide of formula IV is preferably employed in the form of acid addition salt, a base as defined before is preferably added to the

reaction mixture. The total amount of base will depend on the number of salified aminic groups of serinamide; as a general rule, if "n" is the number of equivalents of salified aminic groups, then about "n-1" equivalents of base are added.

Examples of said bases are, as above, tertiary organic aliphatic or alicyclic amines such as trimethylamine, triethylamine (TEA), N-methyl pyrrolidine or heterocyclic bases such as picoline, and the like, preferred being TEA.

The reaction temperature will vary considerably depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures of from 15°C to 30°C, conveniently at room temperature.

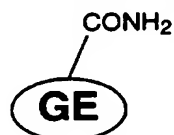
Also the reaction time varies considerably depending on the other reaction parameters; in general the condensation is completed in about 20-40 h.

Generally, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art. On the basis of the results of these assays a man skilled in the art will be able to evaluate the reaction course and decide when to stop the reaction and start working up the reaction mass according to known per se techniques, which include, as above, extraction with solvents, precipitation by pH modification, precipitation by addition of non-solvents, etc., in conjunction with further chromatographic separations and purifications techniques, such as flash chromatography (e.g. on silica gel using dichloromethane/methanol mixtures as eluent), reverse phase chromatography or chromatography on neutral aluminium oxide (using dichloromethane/methanol mixtures as eluent).

A suitable method for preparing the starting material of formula V is as described in European Patent Application no. 565567, here incorporated by reference.

- 5 Antibiotic GE 2270 factor A<sub>2</sub> (prepared as described in the above cited US 5139778), or the corresponding derivatives of GE2270 factor B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub> or amythiamicin factor A, is reacted with ammonia in the presence of an organic protic solvent, preferably (C<sub>1</sub>-C<sub>4</sub>)alcohol, particularly preferred being methanol. After about 2 to 10 4 days, preferably 3 days, the solution is evaporated and the residue is worked up according to the above known per se techniques, thus obtaining the respective amide derivative of formula:

15



- 20 The obtained compound is in turn reacted with a solution of Burgess reagent in an organic aprotic solvent. Suitable solvents are cyclic or glycol ethers such as THF or dioxane or chlorinated solvents such as dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) or chloroform, or mixtures 25 thereof; preferably a mixture of THF/CH<sub>2</sub>Cl<sub>2</sub> is employed.

Furthermore, a base is optionally added to the reaction mixture, as previously described; preferably triethylamine is employed.

- 30 Optionally, further Burgess reagent may be added to the reaction mixture after 12 to 20 hours, preferably after 16 hours.

35

The reaction temperature, depending on the other reaction parameters, may vary from 18°C to 30°C, preferably at room temperature.

Also the reaction time varies considerably depending  
5 on the other reaction parameters; in general the reaction is completed in about 12 to 36 hours after the last addition of Burgess reagent.

Generally, the reaction course is monitored by TLC  
10 or preferably by HPLC according to methods known in the art. On the basis of the results of these assays a man skilled in the art will be able to decide when to stop the reaction and start working up the reaction mass according to known per se techniques, which include, as  
15 above, extraction with solvents, precipitation by pH modification, precipitation by addition of non-solvents, etc., in conjunction with further chromatographic separations and purifications techniques, such as flash chromatography (e.g. on silica gel using  
20 dichloromethane/methanol mixtures as eluent).

The corresponding nitrile derivative of formula



is thus obtained, which is then dissolved in ethanol, preferably in the presence of a chlorinated co-solvent (e.g. dichloromethane, chloroform), and the  
30 solution is cooled at about 0°C; dry HCl is then bubbled through the solution for from 4 to 8 hours, preferably for 6 hours.

The reaction mixture is preferably allowed to stay  
35 at about 4°C for from 10 to 18 hours, and then poured

into a buffering basic solution for neutralizing the excess of HCl; such solution, having a pH lower than 10, is generally a phosphate or carbonate buffer, preferably a carbonate buffer, particularly preferred being a saturated aqueous solution of sodium carbonate.

The solid which precipitates is worked up according to the above known per se techniques, thus obtaining the desired starting material of formula V.

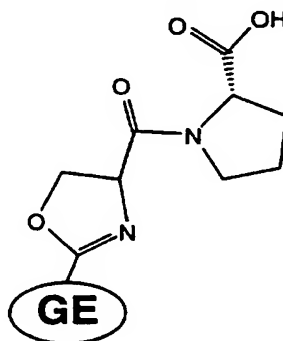
10

A further method for preparing the compounds of the invention (hereinafter defined as "Method C") is to react a compound of formula VI

15

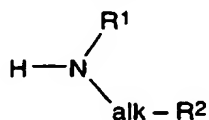
VI

20



wherein the group **GE** is as defined in formula I, with an amine of general formula IVa:

25



IVa

wherein  $\text{R}^1$ , alk, and  $\text{R}^2$  are as defined in formula I, in the presence of an inert organic solvent and of a condensing agent.

30

Useful inert organic aprotic solvents are as defined for method A.

35

Also type and amounts of condensing agent are those defined for the condensation reaction of method A.

The starting material of formula VI is preferably used in its salified form, preferably as an alkali metal salt, particularly preferred being the sodium salt. Thus, a strong acid is conveniently added to the reaction mixture, for freeing the compound from its salt; in general a 2 fold excess of acid equivalents are preferably added. Examples of strong acids are hydrohalide acids or sulfuric acid; preferred being hydrochloric acid.

As above, a salt-forming base is preferably added to the reaction mixture; type and amount of such base will vary depending on the parameters defined above (i.e. amount of reacting amine and use of salified amine), as well as on the presence of the above defined strong acid; if said acid is present, at least an equivalent amount of base for each equivalent of acid is further added to the reaction mixture.

The reaction temperature will vary considerably depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures between 15° and 30°C, conveniently at room temperature.

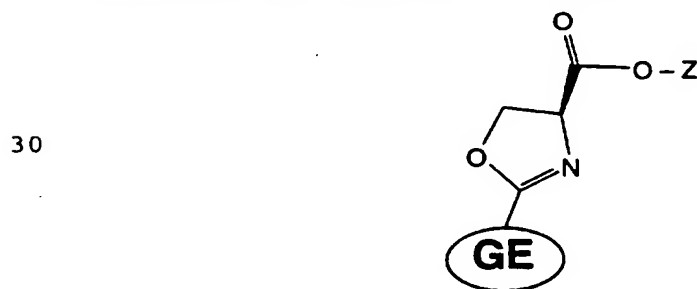
Also the reaction time varies considerably depending on the other reaction parameters. In general the condensation reaction is completed in about 10-16 h.

Generally, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art. On the basis of the results of these assays a man skilled in the art will be able to evaluate the reaction course and decide when to stop the reaction and start

working up the reaction mass according to known per se techniques, which include, as above, extraction with solvents, precipitation by pH modification, precipitation by addition of non-solvents, etc., in  
5 conjunction with further chromatographic separations and purifications techniques, such as flash chromatography (e.g. on silica gel using dichloromethane/methanol mixtures as eluent), reverse phase chromatography or  
10 chromatography on neutral aluminium oxide (using dichloromethane/methanol mixtures as eluent).

A suitable method for preparing the starting material of formula VI is to react a solution of the starting material of general formula V in ethanol,  
15 preferably in the presence of a chlorinated co-solvent (e.g. dichloromethane, chloroform), with a L-serine ( $C_1$ - $C_4$ )alkyl ester salt, preferably methyl ester hydrochloride. The reaction temperature will vary from  $15^{\circ}\text{C}$  to  $30^{\circ}\text{C}$ , preferably about room temperature, for a  
20 time reaction of from 3 to 5 days, preferably about 4 days.

The reaction mixture is then worked up according to known per se techniques, and the solid obtained is purified by means of known chromatographic techniques,  
25 preferably by chromatography on silica gel, thus obtaining the compound of formula:



35 wherein Z represents ( $C_1$ - $C_4$ )alkyl.

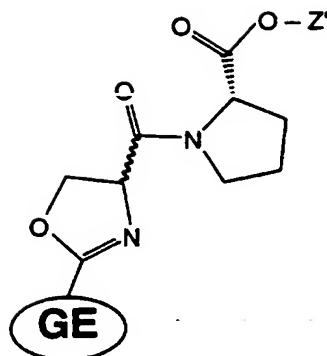
The above compound is then dissolved in an inert organic solvent (e.g. alkylamides, alkylnitriles, saturated linear or cyclic ethers, glycol ethers, phosphoramides, chlorinated solvents or mixtures thereof; preferably dioxane) and hydrolyzed with a strong base, such as an alkali or alkaline-earth metal hydroxide, preferably sodium hydroxide, obtaining the corresponding carboxylic acid sodium salt, which may be recovered according to known per se techniques, for instance by addition of non-solvents, preferably ethyl ether.

The obtained compound, which after the hydrolysis is now a mixture of the two epimers, is then reacted with L-proline (C<sub>1</sub>-C<sub>4</sub>)alkyl ester, preferably methyl ester, in an inert aprotic organic solvent in the presence of a condensing agent; organic solvent and condensing agent are as previously defined in the condensation reaction of method A.

Optionally, a salt-forming base and a strong acid as above defined are also added to the reaction mixture. The reaction temperature will vary from 15°C to 30°C, preferably about room temperature, for a time reaction of from 10 to 16 hours. The reaction mixture is then worked up according to known per se techniques and the solid obtained is purified by means of known chromatographic techniques, preferably by means of flash chromatography, thus obtaining the compound of formula wherein Z' represents (C<sub>1</sub>-C<sub>4</sub>)alkyl.

The obtained compound is finally dissolved in an organic solvent (e.g. alkylamides, alkylnitriles, saturated linear or cyclic ethers, glycol ethers,





phosphoramides, chlorinated solvents or mixtures thereof; preferably dioxane) and hydrolyzed with a strong base, such as an alkali or alkaline-earth metal hydroxide, preferably sodium hydroxide, obtaining the corresponding carboxylic acid sodium salt, which may be recovered according to known per se techniques, for instance by addition of non-solvents, preferably ethyl ether.

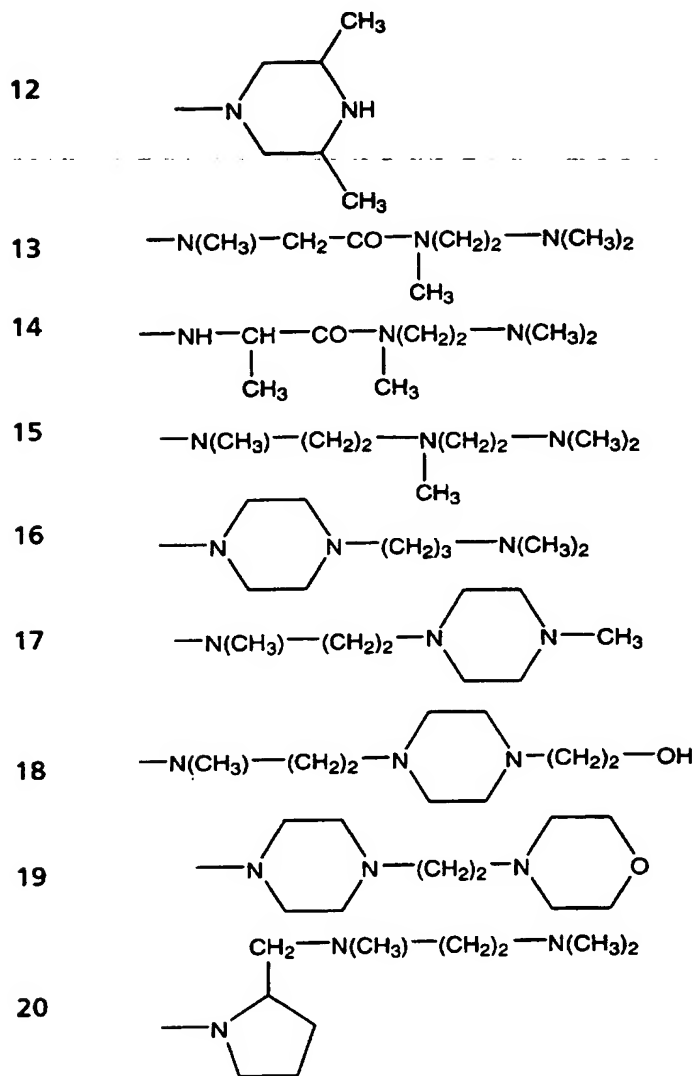
The so obtained starting material is in general a mixture of two epimers. This mixture may be separated or employed as such for the condensation reaction with the amine, thus obtaining an epimeric mixture of the compounds of the invention.

If desired, the epimeric mixture may be separated (either before or after the condensation reaction) according to known per se techniques, such as by reverse phase HPLC, chromatography on neutral or basic aluminium oxide or HPLC on chiral phases.

The following table lists the structural formula of some representative compounds of the invention, for which antimicrobial activity and preparation methodology are given in the following of the specification. The core portion, i.e. the group **GE**, of all the compounds corresponds to antibiotic GE2270 factor A. All the compounds are intended as enantiomeric mixtures (R,S enantiomers), except compound 3s, which corresponds to the S enantiomer.

Compound No.	$\begin{array}{c} \text{R}^1 \\ \diagup \\ \text{---N} \\ \diagdown \\ \text{alk - R}^2 \end{array}$ group
1	
2	
3 and 3s	$\text{---NH---(CH}_2\text{)}_2\text{---N(C}_2\text{H}_5\text{)}_2$
4	$\text{---NH---(CH}_2\text{)}_3\text{---N(CH}_3\text{)}_2$
5	$\text{---N(CH}_3\text{)---(CH}_2\text{)}_2\text{---N(CH}_3\text{)}_2$
6	
7	$\text{---NH---(CH}_2\text{)}_2\text{---N(CH}_3\text{)}_2$
8	$\text{---N(C}_2\text{H}_5\text{)---(CH}_2\text{)}_2\text{---N(CH}_3\text{)}_2$
9	$\text{---N(C}_2\text{H}_5\text{)---(CH}_2\text{)}_2\text{---N(C}_2\text{H}_5\text{)}_2$
10	
11	$\text{---NH---(CH}_2\text{)}_2\text{---N(CH(CH}_3\text{)}_2\text{)}_2$

Compound  
No.



The antimicrobial activity of the compounds of the invention can be demonstrated by a series of standard tests in vitro.

5

The minimal inhibitory concentration (MIC) has been determined by microbroth dilution methodology, in the presence of 0,01% (w/v) of bovine serum albumin (BSA). BSA is added to the diluent to avoid possible adherence of the compounds of the invention to the plastic surface of the microtiter wells, as disclosed also by B. Goldstein et al., Antimicrobial Agents and Chemotherapy, 37 (1993), 741-745.

Inocula were  $10^4$  CFU/ml, except for Propioni-  
15 bacterium acnes and Bacteroides fragilis ( $10^5$  CFU/ml).

MICs were read after 18-24 h, except for Haemophilus influenzae, P. acnes, B. fragilis (48 h).

All microorganisms were incubated at 37°C; H. influenzae in a 5% CO<sub>2</sub> atmosphere, anaerobes in a N<sub>2</sub>-  
20 CO<sub>2</sub>-H<sub>2</sub> (80:10:10) mixture; other organisms in air.

The growth media are: Oxoid Iso-Sensitest broth for staphylococci and Enterococcus faecalis; Difco Todd Hewitt broth for streptococci; Difco brain heart infusion broth + 1% Difco Supplement C for H. influenzae;  
25 influenzae; Difco Wilkins-Chalgren broth for anaerobes.

MICs for some microorganisms are reported below in Table I.

30

35

TABLE I

STRAIN	Internal code	MIC of the compounds ( $\mu\text{g/ml}$ )									
		1	2	3	3s	4	5	6	7	8	9
<i>Staphylococcus aureus</i>	L165	0.06	0.06	0.03	0.06	0.06	0.06	0.13	0.06	0.06	0.06
<i>Staph. epidermidis</i> ATCC 12228	L147	n.t.	n.t.	0.13	0.13	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Staph. haemolyticus</i>	L602	n.t.	n.t.	0.13	0.13	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Streptococcus pyogenes</i>	L49	0.5	0.5	0.5	0.25	4	1	2	1	4	8
<i>Strept. pneumoniae</i>	L44	0.13	0.13	0.13	0.13	0.25	0.25	0.25	0.13	0.25	0.5
<i>Strept. sanguis</i>	L1721	0.25	0.25	0.25	0.13	0.5	0.25	0.5	0.5	0.5	2
<i>Strept. agalactiae</i>	L310	n.t.	n.t.	n.t.	0.5	4	n.t.	n.t.	4	8	8
<i>Enterococ. faecalis</i> ATCC 7080	L149	n.t.	n.t.	0.03	0.06	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Propionibact. acnes</i> ATCC 6919	L1014	n.t.	n.t.	0.03	0.03	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Bacteroides fragilis</i> ATCC 25285	L1011	n.t.	n.t.	8	8	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Haemoph. influenzae</i> ATCC 19418	L970	>16	>16	8	8	n.t.	128	128	n.t.	n.t.	n.t.

STRAIN	Internal code	MIC of the compounds ( $\mu\text{g/ml}$ )																	
		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
<i>Staphylococcus aureus</i>	L165	0.13	0.06	0.06	0.13	0.13	0.06	0.13	0.06	0.06	0.06	0.13							
<i>Staph. epidermidis</i> ATCC 12228	L147	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.							
<i>Staph. haemolyticus</i>	L602	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.							
<i>Streptococcus pyogenes</i>	L49	4	4	2	4	2	8	4	4	4	8	4							
<i>Strept. pneumoniae</i>	L44	0.25	0.25	0.25	0.5	0.13	1	0.5	0.5	0.5	0.5	0.25							
<i>Strept. sanguis</i>	L1721	0.5	0.5	0.25	1	0.25	2	1	0.5	2	2	1							
<i>Strept. agalactiae</i>	L310	8	4	2	8	4	16	8	8	8	16	8							

Compound 3s has also been tested on a number of clinical isolates. Against staphylococci isolates, the MIC was never higher than 0.5 µg/ml; against streptococci isolates, the MIC was never higher than 1.0 µg/ml; 5 against enterococci isolates, the MIC was never higher than 0.13 µg/ml.

The antimicrobial activity of the compounds of the 10 invention is confirmed also in experimental septicemia in the mouse.

Control and treatment groups contain ten CD-1 mice (Charles River) weighing 18-22 g.

Two groups of immunocompetent mice are infected 15 intraperitoneally with 0.5 ml of bacterial suspension prepared by diluting an overnight culture of S. aureus (L819) or S. pyogenes (L49) with sterile peptonized saline, while one group of neutropenic mice is infected intraperitoneally with 0.5 ml of bacterial suspension 20 prepared by diluting an overnight culture of or E. faecalis (L1139) with sterile peptonized saline.

Inocula are adjusted so that untreated animals died of septicemia within 48 h. The compound to be tested is administered intravenously once, immediately after 25 infection with S. aureus, or twice, immediately and 5 hours after infection with S. pyogenes or E. faecalis. On the 7th day, the ED<sub>50</sub> in mg/kg is calculated by the method of Spearman and Kräber (D.J. Finney "Statistical Methods in Biological Assay", Griffin, page 524, 1952) 30 from the percentage of surviving animals at each dose.

The ED<sub>50</sub> (mg/kg/dose) for compound 3s was as follows:

ED<sub>50</sub> for compound 3s in experimental septicemia

Strain	ED <sub>50</sub>
<u>S. aureus</u> (L819)	1.5
<u>S. pyogenes</u> (L49)	7
<u>E. faecalis</u> (L1139)	0.67

5

10 In view of their properties, the compounds of the invention can be used as active ingredients in the preparation of medicaments for human or animal treatment.

15 In particular, the amide derivatives of antibiotic GE 2270 of formula I are antimicrobial agents mainly active against gram positive bacteria.

20 The main therapeutic indication of the antibiotic substances of the invention is thus in the treatment of infections related to the presence of microorganisms susceptible to them.

The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

25 The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine, sheep, poultry and pets in general.

30 The compounds of the invention can be administered as such or in admixture with pharmaceutically acceptable carriers and can also be administered in conjunction with other antimicrobial agents. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compounds in a way that the therapeutic effects of the first administered one is

35

not entirely disappeared when the subsequent is administered.

5 The dosage of the active ingredient depends on many factors which include type, age and conditions of the patient, specific active ingredient and formulation selected for administration, administration schedule, etc.

10 Experimental tests for determining the sensitivity of the microorganisms isolated from the patient may also offer useful indication to select the appropriate dosage.

15 In general, effective antimicrobial dosages are employed per single unit dosage form.

Repeated applications of these dosage forms, e.g. from 2 to 6 times a day, are in general preferred. An effective dosage may be in general in the range 0.5-50 mg/kg body weight/day.

20 Anyway, the prescribing physician will be able to determine the optimal dosage for a given patient in a given situation.

25 The compounds of the invention can be formulated into formulation suitable for parenteral administration containing a liquid vehicle, according to procedures known per se in the art. Examples of suitable vehicles for preparing injectable dosage forms of the compounds of the invention are water, aqueous vehicles (e.g. Dextrose injections), water miscible solvents (e.g. ethyl alcohol, polyethylene glycol, propylene glycol, etc.) and non-aqueous vehicles (e.g. "fixed oils" such as corn oil, cottonseed oil, peanut oil and sesame oil). Optionally, the injectable preparation may further  
35 contain surface-active agent (e.g. polyoxyethylene



sorbitan mono-oleate or polyethoxylated castor oil), buffers for stabilizing the solution (e.g. citrates, acetates and phosphates) and/or antioxidants (e.g. ascorbic acid or sodium bisulfite).

5

For instance, a typical formulation for parenteral administration may contain from 5 to 50 mg of a compound of the invention for ml of final preparation. The compound will generally be formulated in water for  
10 injection, optionally in admixture with 10-20% of a surface-active agent which may be a polyoxyethylene sorbitan fatty acid ester, a polyoxyethylene castor oil derivative or a polyoxyethylene hydrogenated castor oil derivative and 0-20%; optionally, the formulation may  
15 further contain 10-20% of a solubilizing agent such as propylene glycol, dimethylacetamide, dimethylformamide, ter-butyl-N-hydroxycarmabate, 1,2-, 1,3-, or 1,4-butandiol, ethyl oleate, tetrahydrofurfuryl-polyethylene-glycol 200, dimethyl isosorbide, benzyl  
20 alcohol and the like. A preferred solubilizing agent is propylene glycol.

Polyoxyethylene sorbitan fatty acid esters are commercially available and some of them are traded under  
25 the trade name "Tween". They are also known with the non-proprietary name of "polysorbates". Examples of them are polysorbate 20, 21, 40, 60, 61, 65, 80, 81 and 85. Preferred for use in the formulations of the invention is polysorbate 80 (sorbitan mono-9-octadecenoate,  
30 poly(oxy-1,2-ethanediyl)derivatives).

Polyoxyethylene castor oils and polyoxyethylene hydrogenated castor oils are also commercially available. Some of them are traded with the trade name "Cremophor". Examples of such compounds are those known  
35 as Cremophor EL (polyethoxylated castor oil), Cremophor

RH 40 (polyethoxylated hydrogenated castor oil),  
Cremophor RH 60 (PEG 60 hydrogenated castor oil) or  
Emulphor EL-719 (polyoxyethylated vegetable oil).

5        If necessary, the pH of the preparation may be  
adjusted with a suitable buffering agent; conveniently,  
TRIS (i.e. trihydroxymethylaminomethane), phosphate or  
acetate buffers can be used.

10       A particularly preferred formulation for parenteral  
administration is one containing the compound of the  
invention in the salified form dissolved in distilled  
water, without any excipients.

15       An example of such a preparation is the following

Compound 35	50 mg
Water for injection	1 ml
pH 5 with acetic acid	

20       Care should be taken to set the pH at a value of about 5  
for helping the solubilization of the product, but not  
lower than 4.5 because possible hydrolysis of the  
oxazoline ring of the molecule may occur.

25       Examples of formulations of the compounds of the  
invention in admixture with suitable excipients, for  
parenteral administration, are the following:

30    A)	compound 3s	100 mg
	propylene glycol	1 ml
	water for injection q.s.	5 ml
	phosphate buffer pH 8-8.5	

35

B) compound 3s 50 mg  
Cremophor RH 40 1 g  
water for injection q.s. 10 ml  
5 phosphate buffer pH 8-8.5

A further pharmaceutical formulation is represented by a formulation suitable for a topical application on an intact or damaged skin or mucous membrane. Examples of such formulations are powders, ointments, creams and lotions. The excipients in these formulations are the usual pharmaceutically acceptable vehicles such as oleaginous ointment bases (e.g. cetyl esters wax, oleic acid, olive oil, paraffin, spermaceti, starch glycerite); absorbent ointment bases (e.g. anhydrous lanolin, hydrophylic petrolatum), emulsion ointment bases (e.g. cetyl alcohol, glyceryl monostearate, lanolin, stearic acid), water-soluble ointment bases (e.g. glycol ethers and their derivatives which include polyethylene glycols, poly(oxy-1,2-ethanediyl)-alpha-hydro-omega-hydroxy-octadecanoate, polysorbates, and polyethylene glycols mono-stearates).

25 These formulations may contain other known excipients, such as preservatives and are prepared as known in the art and reported in reference handbooks such as Remington's Pharmaceutical Sciences, Seventeenth edition, 1985, Mack Publishing Co.

30

A preferred topical preparation is an ointment containing from 1% to 10% of a compound of the present invention.

35

Besides their use as medicaments in human and veterinary therapy, the compounds of the invention can also be used as animal growth promoters.

5       For this purpose, a compound of the invention is administered orally in a suitable feed. The exact concentration employed is that which is required to provide for the active agent in a growth promotant effective amount when normal amounts of feed are  
10 consumed.

      The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating  
15 the premix into the complete ration.

      Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed.  
20

      The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., S. Francisco, USA, 1969 or  
25 "Livestock Feeds and Feeding" O and B books, Corvallis, Oregon, USA, 1977).

      For better illustrating the invention, the following examples are given.  
30

**EXAMPLES**

METHOD A - Reaction of GE2270 factor A<sub>3</sub> (see preparation no. 3) with L-serinamide (see preparation no. 19) and subsequent cyclization

Example A1:

## Preparation of compound 3s

10

To a solution of GE2270 factor A<sub>3</sub> (1 mmol) in DMF (10 ml) and TEA (2.2 mmol), DPPA (1.2 mmol) is added with stirring at 0°C. The temperature is allowed to rise to room temperature and after 4.5 h a solution of the hydrochloric salt of the selected L-serinamide prepared according to preparation 19 (1.2 mmol) and TEA (3 mmol) in DMF (3 ml) is added with stirring. The reaction is allowed to stir overnight at room temperature and then poured in aqueous 0.06 M NaHCO<sub>3</sub> (200 ml). The precipitate is collected by filtration, allowed to dry in the air and then purified by flash chromatography on silica gel 60 (400-230 mesh) using CH<sub>2</sub>Cl<sub>2</sub> containing from 4% to 10% MeOH as the eluant. For facilitating elution, TEA from 0.1% to 1% (v/v) can be added to the eluant. Fractions containing the condensation product are combined and the solvent evaporated. Thorough washing of the obtained solid with ethyl ether yields the condensation product as a fine powder.

30

A solution of methoxycarbonylsulfamoyl-triethylammonium hydroxide, inner salt (Burgess reagent) (5 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (3 ml) is added dropwise in an argon atmosphere at room temperature over 6 h to a stirred solution of the above condensation product (1 mmol) in dry tetrahydrofuran (THF) (30 ml). At the

35

end of the addition of the Burgess reagent, the disappearance of the condensation product and the formation of a more hydrophilic adduct is controlled by HPLC; then, isopropanol (30 ml) is added to quench  
5 the excess of reagent. Stirring is continued for 2 h at room temperature and then the reaction mixture is refluxed (at about 70°C) for 6 h to cyclize the oxazoline ring. After evaporation of the solvent under reduced pressure, the crude reaction mixture is purified  
10 on neutral aluminum oxide grade I (Merck) using from 2.5% to 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> as the eluant. Fractions containing the title compound are combined and the solvent evaporated to dryness under reduced pressure to yield a solid which is further purified by flash  
15 chromatography on silica gel 60 (400-230 mesh) using CH<sub>2</sub>Cl<sub>2</sub> containing from 4% to 10% MeOH as the eluant. For facilitating elution, TEA from 0.1% to 1% (v/v) can be added to the eluant. Fractions containing the title compound are combined and the solvent evaporated.  
20 Thorough washing of the solid with ethyl ether yields the title compound as a fine powder.

METHOD B - Reaction of starting material GE III (see  
25 preparation no. 6) with L-serinamide (see preparation No. 19)

Example B1:

Preparation of compound 3s

30 To a solution of starting material GEIII (1 mmol) in absolute ethanol (35 ml), CH<sub>2</sub>Cl<sub>2</sub> (3.5 ml) and TEA (3 mmol), L-serinamide prepared according to preparation 19 (3 mmol) is added with stirring at room temperature.  
35 After about 30 h, the reaction mixture is poured in

aqueous 0.06 M  $\text{NaHCO}_3$  (100 ml) and the solid formed is isolated by centrifugation, washed with more water and then taken up in  $\text{CH}_2\text{Cl}_2$  containing few drops of methanol. The solution is dried over  $\text{Na}_2\text{SO}_4$  and the solvent evaporated under reduced pressure to yield a solid which is chromatographed on neutral aluminum oxide grade I (Merck) using from 2.5% to 5% MeOH in  $\text{CH}_2\text{Cl}_2$  as eluant. Fractions containing the title compound are combined and the solvent is evaporated to dryness under reduced pressure to yield a solid which is further purified by flash chromatography on silica gel 60 (400-230 mesh) using  $\text{CH}_2\text{Cl}_2$  containing from 4% to 10% MeOH as eluant. For facilitating elution, TEA from 0.1% to 1% (v/v) can be added to the eluant. Fractions containing the title compound are combined and the solvent evaporated. Thorough washing of the obtained solid with ethyl ether yields the title compound as a fine powder.

20 METHOD C - Reaction of starting material GE VII (see preparation no. 10) with the selected amine

Example C1:

Preparation of compound 3 (epimers mixture)

25

To a stirred solution of the sodium salt of compound GE VII (1 mmol) in DMF (30 ml), TEA (4 mmol) and aqueous 1N HCl (2 mmol) are added at room temperature. After a couple of minutes, the selected amine (1.5 mmol) and DPPA (1.2 mmol) are added thereto and stirring is continued overnight. The reaction mixture is then poured into water (150 ml) and the solid which forms is isolated by centrifugation, washed with water and then taken up in  $\text{CH}_2\text{Cl}_2$  containing a few drops of methanol. The solution is dried over  $\text{Na}_2\text{SO}_4$  and the solvent

35

- evaporated under reduced pressure to yield a solid which is chromatographed on neutral aluminum oxide grade I (Merck) using from 2.5% to 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> as the eluant. Fractions containing the title compound are
- 5 combined and the solvent evaporated. Thorough washing of the obtained solid with ethyl ether yields the title compound as a fine powder.

Example C2:

- 10 Preparation of compounds 1 to 19 (epimers mixture)

- To a stirred solution of the sodium salt of compound GE VII (0.1 mmol) in DMF (9.7 ml), TEA (0.4 mmol) and aqueous 1N HCl (0.2 mmol) are added at room temperature.
- 15 After a couple of minutes, a 0.2 M DMF\* solution of the selected amine (0.2 mmol) and a 0.12 M DMF solution of DPPA (0.14 mmol) are added at the same temperature and stirring is continued overnight.
- 20 \* If salts (hydrochloride, p-toluensulfonate, trifluoroacetate, etc) of the selected amine are used, 0.2 mmol of the salt of the selected amine is first dissolved in water (0.1 ml), then enough TEA to free the base is added and the volume is finally brought to 1 ml
- 25 with DMF.

30

35



The compounds obtained according to the above examples have been characterized by their HPLC retention times, according to the following methodology:

- Column: RP18 (Merck) 5 m
- 5 - Eluent: Phase A: ammonium formate 0.05M;  
Phase B: acetonitrile
- Gradient: minutes 0 2 15 25  
%B 40 40 80 80
- from 2 to 15 minutes: linear gradient of B in A
- 10 - Flow rate: 0.7 ml/min
- Detection: UV at 254 nm and 310 nm.

Compound	Retention time	Compound	Retention time
1	13.0; 13.9	11	13.2; 14.4
2	12.6; 13.4	12	11.1; 11.4
3	12.5; 13.5	13	10.9
3s	12.5	14	10.5; 10.9
4	12.5	15	13.9
5	12.7; 12.4	16	12.0
6	12.5; 12.7	17	12.4; 13.2
7	11.5; 11.9	18	9.6; 9.9
8	13.4	19	10.9
9	14.0; 14.5	20	14.5; 15.1
10	11.9		

Compounds 3 and 3s have also been characterized by means of <sup>1</sup>H-NMR spectra, FAB-MS spectra and UV spectra; 30 methodologies and data are reported hereinafter.

The <sup>1</sup>H-NMR spectra were recorded with a Bruker AM500 or AMX 600 spectrometer using DMSO-d<sub>6</sub> (hexadeuterodimethylsulfoxide) as solvent (s = singlet,

br = broad singlet, d=doublet, dd=doublet of doublets,  
t=triplet, m=multiplet).

Compound 3

5 <sup>1</sup>H-N.M.R. (DMSO-d<sub>6</sub>) δ(ppm): 0.84(d, 3H); 0.87(d, 3H);  
0.95(br, 6H); 1.35(dd, 1H); 2.25-1.85(m, 5H); 2.47(d,  
3H); 2.58(s, 3H); 2.65-2.30(m, 4H); 2.70(dd, 1H);  
3.38(s, 3H); 3.55-3.00(m, 4H); 3.80(m, 2H); 3.98(dd,  
1H); 4.28(m, 2H); 4.57(dd, 1H); 4.79(dd, 1H), 4.97(s,  
2H); 5.00(dd, 1H); 5.20(dd, 1H); 5.25(m, 2H); 5.30(dd,  
1H); 6.00(d, 1H); 7.40-7.20(m, 7H); 7.75(br, 1H);  
10 8.27(m, 2H); 8.41(m, 2H); 8.53(s, 1H); 8.59(s, 1H);  
8.65(m, 2H); 8.98(d, 1H).

Compound 3s

15 <sup>1</sup>H-N.M.R. (DMSO-d<sub>6</sub>) δ(ppm): 0.85(d, 3H); 0.87(d, 3H);  
0.92(br, 6H); 1.35(dd, 1H); 2.20-1.86(m, 5H); 2.47(d,  
3H); 2.58(s, 3H); 2.63-2.32(m, 4H); 2.71(dd, 1H);  
3.38(s, 3H); 3.52-3.04(m, 4H); 3.79(m, 2H); 4.00(dd,  
1H); 4.28(m, 2H); 4.57(dd, 1H); 4.81(dd, 1H), 4.95(s,  
2H); 5.03(dd, 1H); 5.20(dd, 1H); 5.25(m, 2H); 5.30(dd,  
1H); 6.01(d, 1H); 7.40-7.20(m, 7H); 7.77(br, 1H);  
20 8.27(m, 2H); 8.40(m, 2H); 8.55(s, 1H); 8.57(s, 1H);  
8.65(m, 2H); 8.96(d, 1H).

The FAB-MS spectra were obtained with a triple stage  
quadrupole spectrometer TSQ 700 Finningan.

25 Compound 3 FAB-MS m/z 1389 (MH<sup>+</sup>, 100%);  
Compound 3s FAB-MS m/z 1389 (MH<sup>+</sup>, 100%).

30 The UV absorption spectra were recorded with a  
Perkin-Elmer spectrophotometer Mod. Lamda 16 (200-800  
nm).

Compound 3 UV(MeOH) λ<sub>max</sub>= 310 (E1%, 1 cm. =247.2);  
Compound 3s UV(MeOH) λ<sub>max</sub>= 310 (E1%, 1 cm. =245.6).

PREPARATION OF STARTING MATERIALS

PREPARATION OF ANTIBIOTIC GE2270 STARTING MATERIALS

5

Preparation 1: GE2270 factor A

GE2270 factor A is prepared by fermentation of Planobispora rosea ATCC 53773, as described in US Patent  
10 no. 5202241. Recovery and isolation of the factor are as described therein.

Preparation 2: GE2270 factor A<sub>2</sub>

4'-de[4-[[2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl]-  
15 4,5-dihydro-2-oxazolyl]4'-[[[octahydro-1,4-dioxopyrrolo-  
[1,2-a]pyrazin-3-yl)methoxy]carbonyl] GE2270 factor A

GE2270 factor A<sub>2</sub> is prepared by controlled acid hydrolysis from GE2270 factor A, as described in US  
20 Patent no. 5139778.

Preparation 3: GE2270 factor A<sub>3</sub>

4'-carboxy-4'-de[4-[[2-(aminocarbonyl)-1-pyrrolidinyl]-  
25 carbonyl]-4,5-dihydro-2-oxazolyl] GE2270 factor A

GE2270 factor A<sub>3</sub> is prepared by controlled basic hydrolysis from GE2270 factor A<sub>2</sub>, as described in US Patent no. 5139778.

30 Preparation 4: starting material GE I

4'-(aminocarbonyl)-4'-de[4-[[2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl]-4,5-dihydro-2-oxazolyl] GE2270 factor A

Antibiotic GE 2270 factor A<sub>2</sub> (1 mmol) is dissolved  
35 in a saturated solution of NH<sub>3</sub> in methanol (10 ml). The

solution is allowed to stand for 3 days at room temperature and then is evaporated under reduced pressure. The residue is taken up in methanol (2 ml) and the title compound precipitated with water, filtered and  
5 allowed to dry in air. Thorough washing with ethyl ether yields the title compound (GE I) as a white powder.

Preparation 5: starting material GE II

4'-cyano-4'-de[4-[[2-(aminocarbonyl)-1-pyrrolidinyl]-  
10 carbonyl]-4,5-dihydro-2-oxazolyl] GE2270 factor A

A solution of Burgess reagent (3.5 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (5 ml) is added dropwise over 30 min under an argon atmosphere to a well stirred solution of compound  
15 GE I (1 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (15 ml), dry THF (20 ml) and TEA (2.25 ml) at room temperature. After 16h more Burgess reagent (1 mmol) is added in small portions and stirring is continued at room temperature for further  
24h. The reaction mixture is then evaporated to dryness  
20 under reduced pressure and the crude solid is purified by flash chromatography on silica gel 60 (400-230 mesh) using CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5 as eluant. The title compound is obtained as a white powder.

25 Preparation 6: starting material GE III

4'-de[4-[[2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl]-  
4,5-dihydro-2-oxazolyl]-4'-(ethoxyiminomethyl) GE2270  
factor A

30 Compound GE II (1 mmol) is dissolved in absolute ethanol (80 ml) and CHCl<sub>3</sub> (8 ml). The solution is cooled to 0°C and dry HCl is bubbled through it for 6h. The reaction mixture is then allowed to stand overnight at 4°C and the solvent is evaporated under reduced pressure  
35 to a small volume. The concentrated solution is then

carefully poured in an aqueous saturated solution of  $\text{Na}_2\text{CO}_3$  and the resulting precipitate is centrifuged, washed twice with water and then redissolved in chloroform containing the minimum amount of ethanol to help solubilizing the product. The resulting solution is then transferred into a separatory funnel to remove the aqueous layer. The organic phase is dried over  $\text{Na}_2\text{SO}_4$  and the solvent evaporated under reduced pressure to yield a white solid which is triturated with ether and filtered. The title compound is obtained as a white powder.

Preparation 7: starting material GE IV  
9'-de[[2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl]-9'-(methoxycarbonyl) GE2270 factor A

To a solution of compound GE III (1 mmol) in a mixture of absolute ethanol (35 ml) and  $\text{CH}_2\text{Cl}_2$  (3.5 ml), L-serine methyl ester hydrochloride (1,5 mmol) is added with stirring at room temperature under an argon atmosphere. After 4 days the solvent is evaporated under reduced pressure and the resulting crude is purified by flash chromatography on silica gel 60 (400-230 mesh) using  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5 as eluant. The title compound is obtained as a white powder.

Preparation 8: starting material GE V  
4'-(R,S)-carboxy-4'-de[[2-(aminocarbonyl)-1-pyrrolidinyl]-carbonyl] GE2270 factor A

To a solution of compound GE IV (1 mmol) in dioxane (35 ml), 1N NaOH (2 mmol) is added at room temperature with stirring. After 15 min ethyl ether is added to precipitate the title compound which is collected by

filtration. The sodium salt of title compound is obtained as white powder.

Preparation 9: starting material GE VI

- 5 9'-(R,S)-13'-de-(aminocarbonyl)-13'-(methoxycarbonyl)  
GE2270 factor A

- To a solution of the sodium salt of compound GE V of  
(1 mmol) in DMF (25 ml), TEA (4 mmol), aqueous 1N HCl  
10 (0.5 ml), L-proline methyl ester (1.5 mmol) and DPPA  
(1.7 mmol) are added in the order with stirring at room  
temperature. After an overnight stirring, the reaction  
mixture is poured in water (80 ml) and the precipitate  
collected by filtration. The solid is taken up in a  
15 small volume of CH<sub>2</sub>Cl<sub>2</sub> containing a few drops of MeOH to  
help the solubilization of the solid, dried over Na<sub>2</sub>SO<sub>4</sub>  
and the solvent evaporated to dryness under reduced  
pressure. Flash chromatography on silica gel 60 (400-230  
mesh) using CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5 as eluant afforded the  
20 title compound as a white powder.

Preparation 10: starting material GE VII

9'-(R,S)-13'-carboxy-13'-de(aminocarbonyl) GE2270 factor A

- 25 To a solution of compound GE VI (1 mmol) in dioxane  
(30 ml), 1N NaOH (2.3 mmol) is added at room temperature  
with stirring. After 4h ethyl ether is added to  
precipitate the title compound which is collected by  
filtration. The sodium salt of the title compounds  
30 obtained as a pale yellow powder.

PREPARATION OF THE AMINES STARTING MATERIALS

- In the following, it is described the preparation of  
35 particular amines employed as starting materials for

preparing the compounds of the inventions, which amines are not commercially available.

Preparation 11: amine for compound 13

5       A solution of N-Cbz-sarcosine (Novabiochem) (2.0 g, 8.96 mmol), N,N,N'-trimethyl- ethylenediamine (Aldrich) (1.25 ml, 9.86 mmol) and triethylamine (1.40 ml, 9.86 mmol) in dry DMF (30 ml) is stirred at room temperature. DPPA (2.2 ml, 9.86 mmol) is added and  
10       stirring at room temperature is continued for 2 hours. The reaction mixture is poured into water (500 ml), the pH is adjusted to 11 by addition of 1N NaOH and the aqueous phase is extracted with ethyl ether (3x200 ml). The organic phase is dried (MgSO<sub>4</sub>) and concentrated to  
15       dryness. The crude product is purified by flash chromatography on silica gel 60 (400-230 mesh) with methylene chloride/methanol 8/2 to produce pure N,N,N'-trimethylethylenediamine N-Cbz- sarcosinamide as an oil.

      A suspension of the above prepared N,N,N'-trimethyl-  
20       ethylenediamine N-Cbz-sarcosinamide (2.0 g, 6.51 mmol) and 10% palladium on charcoal (200 mg) in methanol (40 ml) is hydrogenated at room temperature and under atmospheric pressure for 1 hour. The catalyst is then removed by filtration and concentration of the solvent  
25       produced the pure unprotected N,N,N'- trimethyl-ethylenediamine sarcosinamide as an oil.

30

35

Preparation 12: amine for compound 14

A solution of N-Boc-L-alanine N-hydroxy-succinimide ester (Novabiochem) (2.0 g, 7 mmol) and N,N,N'-trimethylethylenediamine (Aldrich) (1.0 ml, 7.7 mmol) in dry DMF (30 ml) is stirred at room temperature overnight and then poured into water (600 ml), the pH is adjusted to 9 with sodium carbonate and the aqueous phase is extracted with ethyl ether (2x400ml). The organic phase is dried (MgSO<sub>4</sub>) and the solvent is evaporated in vacuo to yield N,N,N'-trimethyl-ethylenediamine N-Boc-L-alaninamide as a colourless oil.

The above prepared N,N,N'-trimethylethylenediamine N-Boc-L-alaninamide (1.7 g, 6.23 mmol) is dissolved at 0°C in anhydrous TFA (10 ml), then stirred for 5 minutes. After concentration of the solvent at low temperature in vacuo and various washings of the oily product with ethyl ether, the crude trifluoroacetate salt is dissolved in water (10 ml), the pH of the aqueous solution is adjusted to 11 with 1N NaOH and then the product is extracted with CH<sub>2</sub>Cl<sub>2</sub> (2x20 ml). The organic phase is dried (MgSO<sub>4</sub>) and concentrated to dryness in vacuo to give pure N,N,N'-trimethylethylenediamine L-alaninamide trifluoroacetic salt as a gummy oil.

Preparation 13: amine for compound 15

A solution of N,N,N'-trimethylethylenediamine-sarcosinamide (see preparation 11) (750 mg, 4.33mmol) in dry THF (15 ml) is stirred under argon at room temperature. Lithium aluminum hydride (495 mg, 13 mmol) is added in one portion, the temperature is brought to reflux and reflux is continued for additional 6 hours. After cooling to 0°C ethyl acetate (1.5 ml) and 2.5M NaOH (6 ml, 1.2 equivalents) are carefully added



followed by solid  $\text{MgSO}_4$ . The suspension is stirred at room temperature for 15 minutes and then filtered. After concentration of the solvent pure N-(2-dimethylaminoethyl)-N-(2-methylaminoethyl)-methylamine  
5 is obtained as an oil.

Preparation 14: amine for compound 16

1-Benzylpiperazine (Aldrich) (9 ml, 50 mmol) and potassium carbonate (14 g, 0.1 mole) are added at room  
10 temperature to a stirred solution of 3-dimethylaminopropyl chloride hydrochloride (Aldrich) (15.8 g, 0.1 mol) in absolute ethanol (300 ml). The reaction mixture is refluxed for 6 hours, the solvent is evaporated in vacuo and water (300 ml) is added to the  
15 resulting oil. After extraction with  $\text{CH}_2\text{Cl}_2$  (200 ml), the organic phase is washed with water (200 ml), dried ( $\text{MgSO}_4$ ) and the solvent is evaporated in vacuo to yield the 1-benzyl-4-substituted piperazine as an oil.

A suspension of the above prepared 1-benzyl-4-substituted piperazine (9.0 g, 35 mmol) and 10%  
20 palladium on charcoal (3 g) in 95% ethanol (300 ml) is hydrogenated at room temperature and under atmospheric pressure for 6 hours. The catalyst is filtered off and the solution is concentrated to dryness under reduced  
25 pressure to yield the debenzylated product as an oil.

Preparation 15: amine for compound 17

The reaction is carried out as reported in preparation 11 by condensing N-Cbz-sarcosine  
30 (Novabiochem) (2.0 g, 8.96 mmol) with 1-methylpiperazine (Aldrich) (986 mg, 9.86 mmol) and then removing the Cbz-protecting group to yield the expected compound as an oil that is then reduced with lithium aluminum hydride as described in preparation 13 to yield the expected  
35 triamine as an oil.

Preparation 16: amine for compound 18

The reaction is carried out as reported in preparation 11 by condensing N-Cbz-sarcosine  
5 (Novabiochem) (2.0 g, 8.96 mmol) with N-(2-hydroxyethyl)piperazine (Aldrich) (1.28 g, 9.86 mmol) and then removing the Cbz-protecting group to yield the expected compound as an oil that is then reduced with lithium aluminium hydride as described in preparation 13  
10 to yield the expected triaminealcohol as an oil.

Preparation 17: amine for compound 19

1-(4-morpholinocarbonylmethyl)-piperazine (Acros Chimica) (2.13 g, 10 mmol) is reduced as reported in  
15 preparation 13 to yield the expected triamine as an oil.

Preparation 18: amine for compound 20

To a solution of (S)-(-)-2-pyrrolidone-5-carboxylic acid (Aldrich) (500 mg, 3.87 mmol), N,N,N'-  
20 trimethylethylenediamine (Aldrich) (0.54 ml, 4.26 mmol) and triethylamine (0.60 ml, 4.26 mmol) in dry DMF (5 ml), DPPA (0.95 ml, 4.26 mmol) is added under stirring at room temperature. Stirring is continued for 1 hour, then the mixture is poured into ethyl ether (100 ml).  
25 The solid that precipitated is filtered, washed with additional ethyl ether (20 ml) and allowed to dry in air to yield the expected condensation product as a white solid.

Reduction of the above described compound (560 mg, 2.62 mmol) according to preparation 13 yielded the  
30 expected triamine as an oil.

PREPARATION OF THE SERINAMIDES STARTING MATERIALS

Preparation 19: preparation of the L-serinamide starting material employed in Examples A1 and B1 for preparing compound 3s.

A mixture of Cbz-L-Proline (Novabiochem) (145.0 g, 0.58 mol) and N-hydroxysuccinimide (Aldrich) (66.9 g, 0.58 mol) in EtOAc (1.8 L) is cooled at -5°C with stirring under N<sub>2</sub> atmosphere. To this solution, a solution of DCC (132.1 g, 0.64 mol) in EtOAc (265 ml) is added over 20 min so to maintain the internal temperature at -5°C. The temperature is then allowed to rise to ambient temperature and stirring is continued for additional 3 hours. Precipitated dicyclohexylurea is filtered off and the filtrate is used as such in the next step.

To the above prepared solution of N-Cbz-L-proline N-hydroxysuccinimide ester, N,N-diethyl-ethylenediamine (Aldrich) (67.6 g, 0.58 mol) is added over 15 min while stirring at room temperature. After 18 hours the solid that had formed is filtered off, washed over the filter with EtOAc (300 ml) and the filtrate is extracted with 1.04M HCl (725 ml). The aqueous extracts are cooled by an ice-bath, the pH is brought to 10 by careful addition of 1M NaOH and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (4x730 ml). The organic extracts are combined, dried (MgSO<sub>4</sub>) and the solvent evaporated to dryness under reduced pressure to provide an oil which is diluted with Et<sub>2</sub>O (60 ml) and hexane (2 L) under stirring. After 18 hours at room temperature and 1 hour in an ice-bath, the solid product is filtered off, washed with an ice-cold 9:1 mixture of hexane/Et<sub>2</sub>O (2x200 ml) and air-dried at ambient

temperature to yield N,N-diethylethylene-diamine N-Cbz-L-prolinamide as a white powder.

10% Pd/C (5 g) is charged to a 1-L 3-necked flask  
5 fitted with a magnetic stirrer, thermometer and  
continuous N<sub>2</sub> purge. The catalyst is wetted with water  
(20 ml) and then ammonium formate (13.6 g, 0.22 mol) is  
added in one portion. The mixture is stirred while  
adding a solution of the above prepared N,N-  
10 diethylethylenediamine N-Cbz-L-prolinamide (50 g, 0.14  
mol) in methanol (189 ml) over 20 min. After 30 min the  
reaction is completed, the catalyst is filtered off,  
washed over the filter with additional methanol (4x25  
ml) and the filtrate is evaporated to dryness under  
15 reduced pressure to yield N,N-diethylethylenediamine-L-  
prolinamide as an oil.

A mixture of N-Cbz-L-serine (Novabiochem) (100 g,  
0.42 mol) and pentafluorophenol (Aldrich) (84.7 g, 0.46  
20 mol) in anhydrous DMF (250 ml) is cooled with stirring  
under N<sub>2</sub> to -10°C. To this solution, a solution of DCC  
(95.0 g, 0.46 mol) in anhydrous DMF (125 ml) is added  
over 30 min while keeping the reaction temperature at -  
10°C. The reaction mixture is stirred at -10° to -5°C for  
25 an additional 30 min and then at room temperature for 3  
hours. The reaction mixture is poured into water (3.76  
L). After stirring for 15 min, the solid that  
precipitated out is filtered, washed over the filter  
with water (3x500 ml) and air dried at room temperature.  
30 The solid is then taken up in EtOAc (1 L) and the  
residual solid (mainly dicyclohexylurea) is filtered off  
and washed with more EtOAc (3x150 ml). The combined  
EtOAc solutions are evaporated to dryness under reduced  
pressure.

The residual solid is dissolved in hot  $\text{CH}_2\text{Cl}_2$  (3.2 L). The hot solution is gravity filtered and the solvent is boiled off until solid began to crystallize. The solid which crystallized is filtered and air dried to ambient temperature to give N-Cbz-L-serine pentafluorophenyl ester as a white solid.

A solution of N,N-diethylethylenediamine-L-prolinamide (63.1 g, 0.30 mol) in  $\text{CH}_2\text{Cl}_2$  (500 ml) is charged to 2-L 3-necked flask fitted with a magnetic stirrer, thermometer and continuous  $\text{N}_2$  purge. The solution is stirred while adding N-Cbz-L-serine pentafluorophenyl ester (121.6 g, 0.30 mol) as a solid over 10 min. The reaction mixture is stirred for an additional 1 hour at room temperature and then is washed with 1N NaOH (105 ml followed by 2x210 ml). The organic phase is separated, dried ( $\text{MgSO}_4$ ) and then evaporated to dryness under reduced pressure. The glassy product is diluted with  $\text{Et}_2\text{O}$  (200 ml) and the mixture is warmed to  $30^\circ\text{C}$  and solid began to form. After all of the glass had solidified, the mixture is diluted with pentane (200 ml). Solid is filtered off, washed with pentane, then air-dried at room temperature to give a solid which is slurried in  $\text{Et}_2\text{O}$  (250 ml). Solid is filtered off and allowed to air-dry at room temperature to give the expected N-Cbz-L-serinamide as a white powder.

Deprotection of the Cbz-protecting group is carried out just before usage of the serinamide.

A suspension of the above prepared N-Cbz-L-serinamide (5.0 g, 11.52 mmol) and 10% palladium on charcoal (500 mg) in methanol (90 ml) is hydrogenated at room temperature and atmospheric pressure in the presence of 20% methanolic HCl (4.6 ml) for 1 hour. The catalyst is filtered off, washed over the filter with

methanol (2x100 ml) and the solvent evaporated to dryness under reduced pressure. Trituration of the waxy solid with Et<sub>2</sub>O yielded the expected scorinamide hydrochloric salt as a white powder.

5

10

15

20

25

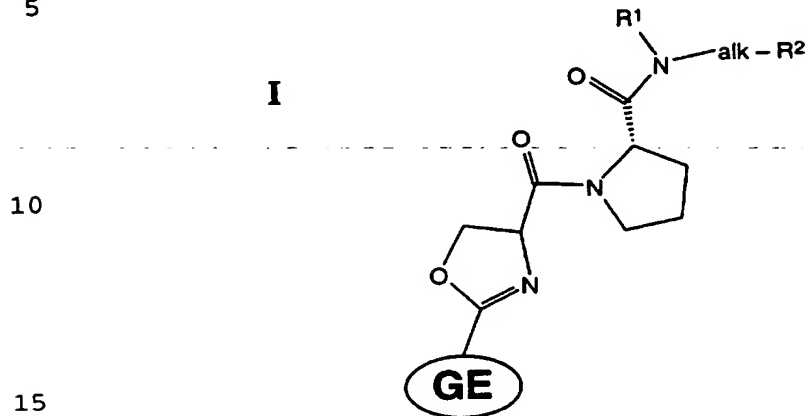
30

35

## CLAIMS

1. Basic amides derivatives of GE 2270 and GE 2270-like antibiotics of general formula I

5



wherein:

20 R<sup>1</sup> represents hydrogen, (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene,

alk represents (C<sub>1</sub>-C<sub>4</sub>)alkylene or (C<sub>2</sub>-C<sub>5</sub>)alkylene-carbonyl,

25 R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

R<sup>3</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene, or di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene, and

30 R<sup>4</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene or hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene,

or a five or six membered heterocycle ring containing one nitrogen atom and optionally a further heteroatom selected from nitrogen and oxygen, optionally substituted with a group selected

35

from (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene,  
di(C<sub>1</sub>-C<sub>4</sub>)alkylamino and di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-  
(C<sub>1</sub>-C<sub>4</sub>)alkylene;

5 or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen  
atom form a five or six membered heterocycle ring  
optionally containing a further heteroatom selected from  
oxygen and nitrogen, optionally substituted with a group  
selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, di(C<sub>1</sub>-  
10 C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene, hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene and  
a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>4</sub>)alkyl and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

15 R<sup>6</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-  
amino(C<sub>1</sub>-C<sub>4</sub>)alkylene and

R<sup>7</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-  
amino(C<sub>1</sub>-C<sub>4</sub>)alkylene

20 or a five or six membered heterocycle ring  
containing one or two heteroatoms selected from  
nitrogen and oxygen, optionally substituted with  
a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl, hydroxy(C<sub>1</sub>-  
C<sub>4</sub>)alkylene, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino and di(C<sub>1</sub>-  
25 C<sub>4</sub>)alkylamino(C<sub>1</sub>-C<sub>4</sub>)alkylene

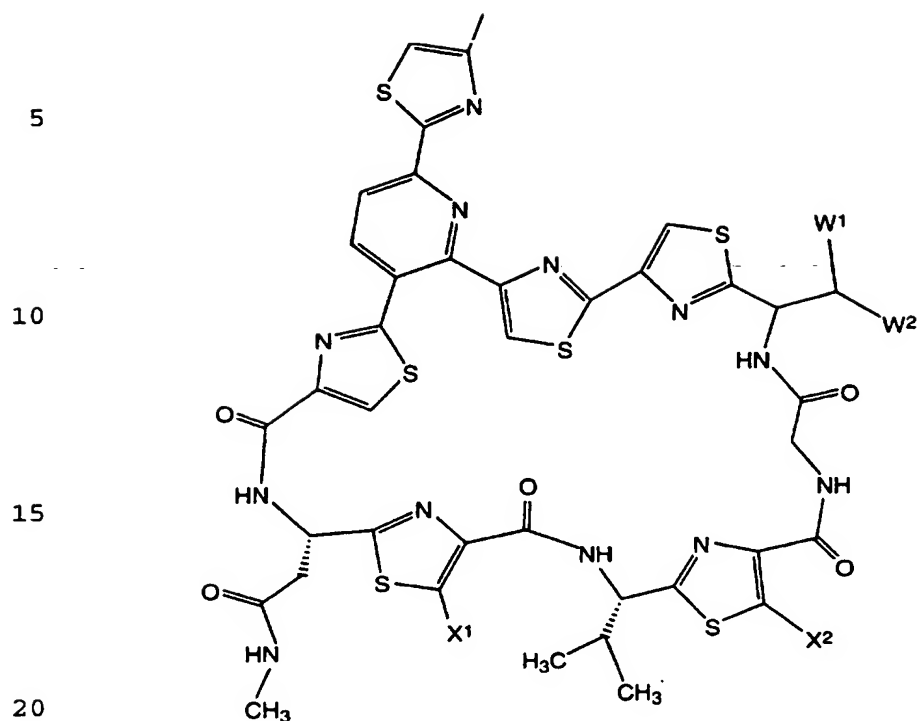
and the group of formula

GE

30 represents the antibiotic core portion of formula

35





wherein:

W<sup>1</sup> represents phenyl

25 W<sup>2</sup> represents hydroxy

or both W<sup>1</sup> and W<sup>2</sup> represent methyl,

X<sup>1</sup> represents hydrogen or methyl,

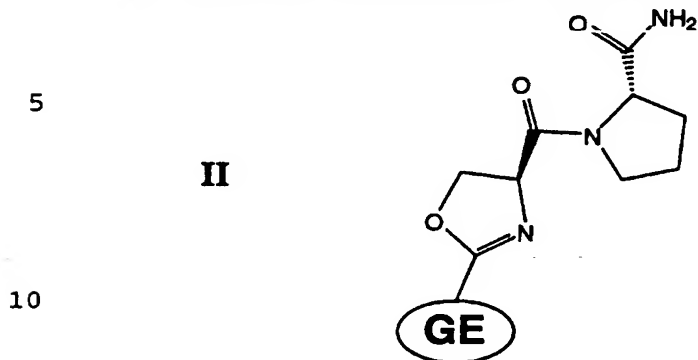
X<sup>2</sup> represents hydrogen, methyl or methoxymethylen,

30 with the proviso that when both W<sup>1</sup> and W<sup>2</sup> are methyl,  
then X<sup>1</sup> is methyl and X<sup>2</sup> is hydrogen,

or a pharmaceutical acceptable salt thereof.

35

2. Compound according to claim 1 of formula



wherein R<sup>1</sup>, alk, R<sup>2</sup> and the group **GE** are as defined in claim 1.

3. Compound according to claim 1 or 2 wherein the group **GE** is as defined in claim 1 and

20 R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>4</sub>)alkyl,

alk represents (C<sub>1</sub>-C<sub>4</sub>)alkylene or (C<sub>2</sub>-C<sub>5</sub>)alkylene-carbonyl,

25 R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

R<sup>3</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl and

R<sup>4</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-(C<sub>1</sub>-C<sub>4</sub>)alkylene,

30 or a five or six membered heterocycle ring containing one or two nitrogen atoms, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>4</sub>)alkyl and hydroxy(C<sub>1</sub>-C<sub>4</sub>)alkylene;

35 or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring

optionally containing a further nitrogen atom,  
optionally substituted with a group selected from  
(C<sub>1</sub>-C<sub>4</sub>)alkyl, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino, di(C<sub>1</sub>-C<sub>4</sub>)alkylamino-  
(C<sub>1</sub>-C<sub>4</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

5       alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkyl and

      R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

          R<sup>6</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl and

          R<sup>7</sup> represents (C<sub>1</sub>-C<sub>4</sub>)alkyl or di(C<sub>1</sub>-C<sub>4</sub>)alkyl-  
              amino(C<sub>1</sub>-C<sub>4</sub>)alkylene

10       or a five or six membered heterocycle ring  
          containing one or two heteroatoms selected from  
          nitrogen and oxygen.

15       4. Compound according to claim 1 or 2 wherein the  
          group **GE** is as defined in claim 1 and

      R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>2</sub>)alkyl,

20       alk represents (C<sub>1</sub>-C<sub>3</sub>)alkylene or (C<sub>2</sub>-C<sub>3</sub>)alkylene-  
          carbonyl,

      R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

          R<sup>3</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl and

25       R<sup>4</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-  
          (C<sub>1</sub>-C<sub>2</sub>)alkylene,

          or a five or six membered heterocycle ring  
          containing one or two nitrogen atoms, optionally  
          substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl  
30       and hydroxy(C<sub>1</sub>-C<sub>2</sub>)alkylene;

          or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen  
          atom form a five or six membered heterocycle ring  
          optionally containing a further nitrogen atom,  
35       optionally substituted with a group selected from

(C<sub>1</sub>-C<sub>2</sub>)alkyl, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-(C<sub>1</sub>-C<sub>2</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkyl and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

5 R<sup>6</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl and

R<sup>7</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkyl-amino(C<sub>1</sub>-C<sub>2</sub>)alkylene

or a five or six membered heterocycle ring

10 containing one or two heteroatoms selected from nitrogen and oxygen.

5. Compound according to claim 1 or 2 wherein the group **GE** is such that W<sup>1</sup> is phenyl, W<sup>2</sup> is hydroxy, X<sup>1</sup> is  
15 methyl, X<sup>2</sup> is methoxymethylene and

R<sup>1</sup> represents hydrogen or (C<sub>1</sub>-C<sub>2</sub>)alkyl,

alk represents (C<sub>1</sub>-C<sub>3</sub>)alkylene,

20

R<sup>2</sup> represents a NR<sup>3</sup>R<sup>4</sup> group wherein

R<sup>3</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl and

R<sup>4</sup> represents (C<sub>1</sub>-C<sub>3</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-(C<sub>1</sub>-C<sub>2</sub>)alkylene,

25 or a five or six membered heterocycle ring containing one or two nitrogen atoms, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl and hydroxy(C<sub>1</sub>-C<sub>2</sub>)alkylene;

30 or R<sup>1</sup> and alk-R<sup>2</sup> together with the adjacent nitrogen atom form a five or six membered heterocycle ring optionally containing a further nitrogen atom, optionally substituted with a group selected from (C<sub>1</sub>-C<sub>2</sub>)alkyl, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino, di(C<sub>1</sub>-C<sub>2</sub>)alkylamino-  
35 (C<sub>1</sub>-C<sub>2</sub>)alkylene and a alk<sub>2</sub>-R<sup>5</sup> group wherein

alk<sub>2</sub> is (C<sub>1</sub>-C<sub>2</sub>)alkylene and

R<sup>5</sup> is a NR<sup>6</sup>R<sup>7</sup> group wherein

R<sup>6</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl and

R<sup>7</sup> represents (C<sub>1</sub>-C<sub>2</sub>)alkyl or di(C<sub>1</sub>-C<sub>2</sub>)alkyl-  
amino(C<sub>1</sub>-C<sub>2</sub>)alkylene

or a five or six membered heterocycle ring  
containing one or two heteroatoms selected from  
nitrogen and oxygen.

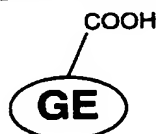
10

6. Process for preparing a compound of claim 1 which  
comprises:

a) reacting a compound of formula III

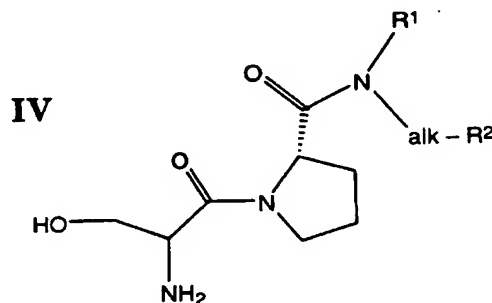
15

III



wherein the group **GE** is as defined in formula I, with a  
serinamide, or an acid addition salt thereof, of formula

20 IV:

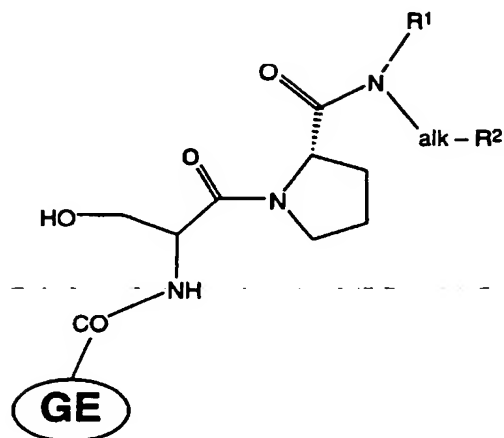


30

wherein R<sup>1</sup>, alk and R<sup>2</sup> are as in claim 1, in an inert  
aprotic organic solvent in the presence of a condensing  
agent;

b) cyclizing the serine moiety of the obtained compound  
of formula IIIa

35



IIIa

15 with a suitable cyclizing reactant, in order to obtain the desired compound of formula I.

7. Process according to claim 6 wherein a salt-forming base is further added to the reaction mixture of step a).

20 8. Process according to claim 6 wherein the salt-forming base is a tertiary organic aliphatic or alicyclic amine or heterocyclic base.

25 9. Process according to claim 6 wherein the inert organic solvent of step a) is selected from organic amides, ethers of glycols and polyols, phosphoramides, sulfoxides and mixtures thereof.

30 10. Process according to claim 9 wherein the inert organic solvent is selected from dimethylformamide, dimethoxyethane, hexamethylphosphoramide, dimethylsulfoxide, dioxane, and mixtures thereof.

35 11. Process according to claim 6 wherein the condensing agent is selected from diphenyl-phosphorazidate,

diethyl-phosphorazidate, di(4-nitrophenyl)-phosphorazidate, dimorpholyl-phosphorazidate, diphenylphosphorochloridate and benzotriazol-1-yl-oxy-tripyrrolidinophosphoniumhexa-fluorophosphate.

5

12. Process according to claim 6 wherein the serine cyclization of step b) is obtained by reacting compound IIIa with methoxycarbonylsulfamoyl-triethylammonium hydroxide, inner salt (Burgess reagent), in an organic aprotic oxygenated solvent and refluxing the reacted mixture.

10

13. Process according to claim 12 wherein the organic aprotic oxygenated solvent is selected from tetrahydrofuran and dioxane.

15

14. Process according to claim 12 wherein a chlorinated solvent is further added to the reaction mixture.

15. Process according to claim 12 wherein a salt-forming base is further added to the reaction mixture.

20

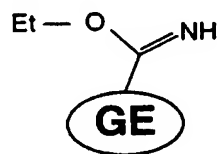
16. Process according to claim 12 wherein a secondary or tertiary (C<sub>3</sub>-C<sub>5</sub>)alcohol is subsequently added to the reaction mixture for quenching the reaction.

25

17. Process for preparing a compound of claim 1 which comprises reacting a compound of formula V

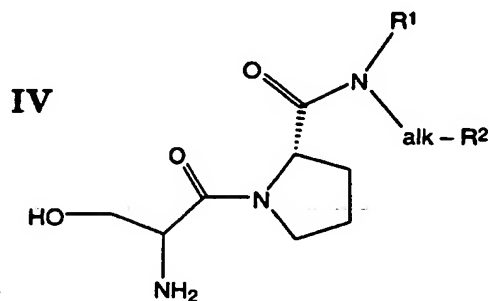
30

V



35

wherein the group **GE** is as defined in claim 1, with a serinamide, or an acid addition salt thereof, of formula IV:



wherein R<sup>1</sup>, alk and R<sup>2</sup> are as in claim 1, in a protic organic solvent.

15

18. Process according to claim 17 wherein a salt-forming base is further added to the reaction mixture.

20

19. Process according to claim 18 wherein the salt-forming base is a tertiary organic aliphatic or alicyclic amine or heterocyclic base.

25

20. Process according to claim 17 wherein the protic organic solvent is selected from methanol, ethanol, propanol, iso-propanol, butanol, iso-butanol and mixtures thereof.

30

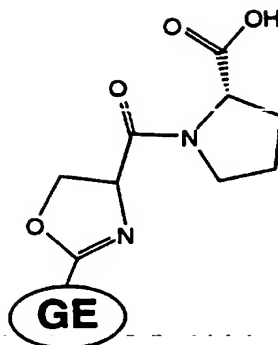
21. Process for preparing a compound of claim 1 which comprises reacting a compound of formula VI

35

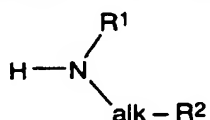


5

VI



- 10 or a base addition salt thereof, wherein the group **GE** is as defined in formula I, with an amine, or an acid addition salt thereof, of general formula IVa:



IVa

wherein R<sup>1</sup>, alk, and R<sup>2</sup> are as defined in formula I, in the presence of an inert organic solvent and of a condensing agent.

- 20 **22.** Process according to claim 21 wherein, when the compound of formula VI is employed in the salified form, a strong acid is further added to the reaction mixture.

- 25 **23.** Process according to claim 21 wherein a salt-forming base is further added to the reaction mixture.

- 24.** Process according to claim 23 wherein the salt-forming base is a tertiary organic aliphatic or alicyclic amine or heterocyclic base.

- 30 **25.** Process according to claim 21 wherein the inert organic solvent is selected from organic amides, ethers of glycols and polyols, phosphoramides, sulfoxides and mixtures thereof.

35

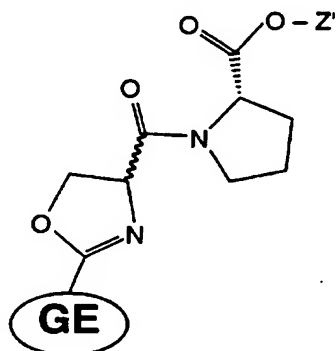
26. Process according to claim 21 wherein the inert  
organic solvent is selected from dimethylformamide,  
dimethoxyethane, hexamethylphosphoramide, dimethyl-  
5 sulfoxide, dioxane, and mixtures thereof.

27. Process according to claim 21 wherein the  
condensing agent is selected from diphenyl-phosphor-  
azidate, diethyl-phosphorazidate, di(4-nitrophenyl)-  
10 phosphorazidate, dimorpholyl-phosphorazidate,  
diphenylphosphorochloridate and benzotriazol-1-yl-oxy-  
tripyrrolidinophosphoniumhexa-fluorophosphate.

28. Compound of formula

15

20



25 wherein Z' represents (C<sub>1</sub>-C<sub>4</sub>)alkyl and **GE** is as defined  
in claim 1.

29. Compound according to any of claims 1, 2, 3, 4 or 5  
for use as a medicament.

30

30. Use of a compound of any of claims 1, 2, 3, 4 or 5  
for preparing an antimicrobial medicament.

35

31. Pharmaceutical composition containing a compound of any of claims 1, 2, 3, 4 or 5 in admixture with a pharmaceutical acceptable carrier.

5

10

15

20

25

30

35

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 96/00407

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07K5/06 A61K38/05 //C07K7/56

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 494 078 (GRUPPO LEPETIT) 8 July 1992 cited in the application * Examples 1-44 *	1
A	J. ANTIBIOT., vol. 47, no. 12, December 1994 pages 1164-115967, XP 000568773 TAVECCHIA, P. ET AL. 'Revised structure of the antibiotic GE 2270' cited in the application * fig. 4 *	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

29 April 1996

Date of mailing of the international search report

17.05.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
T'd. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+ 31-70) 340-3016

Authorized officer

Hermann, R

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/EP 96/00407

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-494078	08-07-92	AT-T- 117320	15-02-95
		AU-B- 662122	24-08-95
		AU-B- 1154492	17-08-92
		CA-A- 2093219	04-07-92
		CN-A- 1063110	29-07-92
		DE-D- 69201236	02-03-95
		DE-T- 69201236	01-06-95
		WO-A- 9212172	23-07-92
		EP-A- 0565567	20-10-93
		ES-T- 2067325	16-03-95
		JP-T- 6504768	02-06-94
		NZ-A- 241216	27-04-94
-----			